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ARTHUR W. GREELY.

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BIOLOGICAL BULLETIN

ARTHUR W. GREELEY.

Arthur W. Greeley died at St. Louis, after an operation for appendicitis, on March 15, 1904, at the age of twenty-eight. The following paper, which he had prepared for publication just before his death, will indicate how lamentable for American science is the loss of this enthusiastic, industrious and keen investigator.

In this suggestive paper, explaining on the basis of modern physical chemistry the changes in structure of protoplasm accompanying changes in function, Greeley has mapped out for others work which he had planned for himself, but which he was unable to accomplish. There has been hitherto no thorough study of the changes in structure of living protoplasm produced by salts from the standpoint of modern views of electrolytes and colloidal solutions. This paper is a most important contribution to this subject and opens up a great field for further work. It was Greeley's good fortune to be able to reduce the changes to order, and thus to supply the structural basis for observed changes in function produced by salts and other agencies. As a result of this work he was able to reduce many of the so-called "tropic" responses of organisms to a common basis; all agencies producing a certain change in the protoplasm producing also a definite response in orientation of the organism. What a great step in advance this is will be appreciated by those familiar with the confusion prevailing in this most difficult field.

This paper, in connection with his earlier discoveries of the production of spores in infusoria by cold; on the identity of the physiological action of dehydration and exposure to low temperatures, and of the production of artificial parthenogenesis in the echinoderm egg by cold, stamps Greeley as an original,

industrious and accurate investigator of great scientific ability, doing work of a most fundamental character. All of those who knew him feel that in his death a man of great promise has passed away.

Dr. Greeley was born in Oswego, New York State, in 1875. He took his undergraduate degree in Stanford University in 1898, and spent one year as a graduate student in zoölogy, during which he went to Alaska with the fur-seal expedition and to Brazil with the Agassiz expedition. The following year he was a teacher in the State Normal School at San Diego, leaving there to enter the University of Chicago as fellow in physiology. Two years later he took his doctorate of philosophy under Loeb with a thesis on the action of low temperatures on the infusoria, and was then appointed Assistant Professor of Zoölogy at the Washington University, in St. Louis. For three summers he was a member of the staff of instruction in physiology at the Marine Biological Laboratory of Wood's Holl. During his two years of residence in St. Louis his enthusiasm and unusual personality had already aroused marked interest in biological science in that city.

Dr. Greeley was of a rare and winning personality, remarkable for extraordinary enthusiasm which inspired all with whom he came in contact. He had a happy disposition, great courage and high principles. His frank open nature, his consideration for others and his loyalty made him many friends; and he had no enemies. He was an inspiring teacher. To his university, to his friends and to his colleagues his sad death at the outset of a most promising career is an irreparable loss.

A. P. MATHEWS.

EXPERIMENTS ON THE PHYSICAL STUCTURE OF THE PROTOPLASM OF PARAMŒCIUM AND ITS RELATION TO THE REACTIONS OF THE ORGANISM TO THERMAL, CHEMICAL AND ELECTRICAL STIMULI.

ARTHUR W. GREELEY.

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INTRODUCTION.

It has been known for several years that a marked similarity in physical structure exists between protoplasm and that class of chemical solutions known as colloidal solutions. This similarity was pointed out by Hardy in 1899 as a result of his investigations upon the physical structure of certain organic colloids, as egg albumen, gelatin, etc., which resemble protoplasm very closely in their gross appearance, and of observations upon protoplasm itself under various conditions.

The conclusions of Hardy and others[1] in regard to the physical structure of the organic colloids are of such importance in the further development of this paper, and are so largely unappreciated by biologists, that they will be briefly recapitulated at this place.

1. A colloidal solution consists of a fluid matrix which holds in suspension more solid or viscous granules (the colloidal par-

[1] Hardy, *Jour. of Physiol.*, 1899, XXIV., p. 172; *ibid.*, p. 288. Mann, "Physiological Histology," Oxford, 1902.

ticles), which differ from the dissolved substance in a crystalloidal solution in that they are relatively very large aggregates of molecules of the colloidal matter. These particles do not affect the osmotic pressure of the fluid matrix; while in a crystalloidal solution the solute exists in a molecular or ionic condition, and thus gives to the solution a definite osmotic pressure.

2. The physical state of the entire solution depends on the condition of these colloidal particles. When they are finely divided and separated from each other in the solvent, the colloid appears as a liquid or exists in the "sol" phase. If, however, the colloidal particles become fused, and thus lose their condition of fine suspension, the colloid becomes relatively solid, or passes into the "gel" phase.

3. The physical state of the colloidal particles and hence of the entire solution varies directly with certain external conditions. Thus the passage from the "sol" into the "gel" phase is accomplished in the following ways: (*A*) by variations in the temperature, (*B*) by chemical changes, (*C*) by the action of the electric current.

A. The physical state of the organic colloids varies directly with the temperature. If the "sol" phase is constant at the normal temperature, 20° C., coagulation gradually takes place as the temperature is lowered, until at 0° C. almost complete gelation has occurred. As the temperature is raised above the normal the fluidity of the solution is increased (by the subdivision of the colloidal particles and absorption of water), until a critical point is reached at which coagulation suddenly occurs, and the colloid goes into a condition of heat rigor.

B. A colloid may be coagulated by adding to it a solution of any electrolyte which bears an electrical charge opposite in sign to that carried by the colloidal particles. Thus Hardy found that a positively charged colloidal solution was coagulated by any electrolyte with a powerful anion or negatively charged ion, and the rapidity of the coagulation varied directly with the valence of the anion. A negatively charged colloid was coagulated by electrolytes of an opposite electrical character, or by powerful cathions. Similarly an electrolyte carrying a charge of the same sign as that of the colloidal particles causes them to subdivide

still further. As a result water is absorbed so that a liquefaction of the whole colloid occurs, as by a slight increase in the temperature. Thus cathions liquefy positively charged colloids; anions, negatively charged colloids.

C. The same conditions prevail in the reaction of colloids to the electrical current. Negatively charged colloidal particles fuse and form a "gel" around the anode, and tend to liquefy about the cathode during the passage of the current. Positively charged colloids coagulate about the cathode, and liquefy about the anode.

From the behavior of these colloidal solutions under various chemical and electrical conditions Hardy concluded, that the "sol" phase is maintained under normal conditions because all the colloidal particles carry an electrical charge of the same sign, and are thus mutually repelled, and remain in a state of fine suspension. Whenever there is introduced an electrical charge of an opposite sign, either by means of a dissociated electrolyte or by the electrical current, the charge carried by the colloidal particles is neutralized, and fusion or coagulation occurs.

The reaction to temperature variations is apparently due to the fact that, within certain limits, the kinetic energy of the colloidal particles varies directly with the temperature. A reduction of the kinetic energy causes a gradual fusion of the particles; an increase brings about a still finer state of suspension, and consequent liquefaction of the colloid through the absorption of water. The sudden coagulation at the critical point is probably due to a chemical change in the colloid itself.

The chief points of similarity between these colloidal solutions and protoplasm made apparent by Hardy's work are as follows:

1. The elementary physical structure of the two is the same. Like the colloid, protoplasm is known to be made up of two substances — (*a*) the fluid cell-sap or matrix which holds in suspension (*b*) the more solid proteid or protoplasmic particles, granules or microsomes of the morphologists. And the physical state of the protoplasm depends on the condition of these protoplasmic granules, just as the state of the artificial colloid depends

on the condition of its constituent particles. It is significant to find, that all the controversies that have been waged over the various theories of a fixed morphological structure in protoplasm have centered about the supposed unchanging physical condition of these more solid or viscous elements in the protoplasm. Now in the artificial colloids we see that their physical state varies directly with certain external conditions. The protoplasmic particles of course are vastly larger than the particles in an artificial colloidal solution, and we have no right to assume that the two are identical. But the protoplasmic granules bear the same relation to the cell-sap as the colloidal particles do to the fluid matrix, and they both respond to chemical and physical changes in a similar manner. A close comparison of the behavior of these two structural elements under various conditions will, I believe, throw a great deal of light on the physical basis of protoplasm.

Hardy observed that when a colloidal solution is exposed to the action of certain so-called fixing agents, the same structures are produced as by the action of these fixing agents upon protoplasm, *viz.:* a coagulation occurs in which the colloidal particles fuse in definite ways, producing a type of structure varying with the fixing agent employed. He therefore concluded that coagulation of the protoplasm is necessary to reveal the fixed types of structure thought by some to be characteristic of protoplasm under all conditions. Such types of structure then appeared to be in protoplasm, as in the organic colloids, merely artefacts; and Hardy argued that protoplasm in the living condition must be identical, as far as its physical structure is concerned, with the organic colloids in the "sol" phase, and that like the colloids its structure is constantly changing with the external conditions. Undoubtedly under the same external conditions, the protoplasm of different forms will show varying types of structure, as the above statement will allow. In some cases this structure may resemble a reticulum or other types described by the morphologists, but no one will now maintain that such a type is of universal occurrence. In the living protoplasm of *Paramœcium*, I have never seen a trace of a fixed structure.

These suggestions of Hardy and others upon the physical structure of protoplasm remained almost unnoticed by biolo-

gists until, very recently, attention was directed to them by the work of Loeb,[1] Mathews,[2] R. S. Lillie and others. Mathews, in particular, in his work upon the chemical stimulation of the motor nerve of the frog, has arrived at the conclusion that the protoplasm of the nerve is a colloidal solution whose particles carry a definite electrical charge, and that stimulation of the nerve is accomplished by a reversible change in the physical state of the protoplasm, analogous to coagulation. He finds that this stimulation or coagulation is effected chemically only by a series of electrolytes which agree in carrying a predominant negative charge of electricity, as would be the case in Hardy's positively charged colloidal solutions. It is only fair to say that these remarkable investigations of Mathews have been the inspiration of this work on the physical structure of protoplasm. R. S. Lillie[3] shows, in his work on the reaction of cytoplasmic and nuclear structures to the electric current, that these forms of protoplasm behave exactly as would positively and negatively charged colloidal solutions under the same conditions.

The experiments described above have shown that protoplasm reacts to various external conditions in a manner strictly parallel to the behavior of colloidal solutions in the presence of like stimuli. But meanwhile we have had but little evidence of the precise structural changes, that accompany these reactions, and are, as we have seen, the leading distinguishing feature of colloidal solutions. It was with the idea of studying these structural changes, which occur in protoplasm when subjected to various external stimuli, and of comparing them with the changes in colloidal solutions under the same conditions, that the following experiments were undertaken.

The material used has been the protoplasm of various protozoa, viz: *Paramœcium*, *Stentor*, *Amœba* and others, which are ideal objects for this study because of the abundance in which

[1] Loeb, as a result of his work on the stimulation of contractile tissue by ions, and on the toxic and antitoxic effect of ions on the duration of life of the *Fundulus* egg and frog's muscle, was the first to suggest, that the protoplasm reacts under these conditions like artificial colloids, although he has elsewhere adopted a different explanation. (See *Amer. Jour. Physiol.*, 1902, VI., p. 411.)

[2] Mathews, *Science*, 1902, XV., p. 492; 1903, XVII., p. 729.

[3] Lillie, *Amer. Jour. Physiol.*, 1903, VIII., p. 273.

they can be procured, and especially because their small size makes it possible to study the structural changes in the living protoplasm under high magnifications, which is out of the ques-question in more bulky tissues. The protozoa were subjected to thermal, chemical and electrical changes, and the structural modifications produced by these means were studied.

Structural Reactions of the Protoplasm of Protozoa to Physical and Chemical Changes.

I. *Reactions to Variations in the Temperature.*

In previous papers[1] I have described the structural changes that occur in the protoplasm of various protozoa when exposed to variations in the temperature, so that a very brief description will suffice here. We find, exactly as in the case of organic colloids, that the fluidity of the protoplasm varies directly with the temperature within certain limits. As the temperature is lowered below the normal, 20°C., a very gradual coagulation occurs, because of the decrease in kinetic energy and consequent fusion of the protoplasmic particles. This change is accompanied by a loss of water, so that at 0° C. there is produced a nearly solid opaque, spherical mass of protoplasm, which exists only in a resting condition. As the temperature is elevated above the normal, the reverse changes occur through the increase in kinetic energy acquired by the protoplasmic particles. They may be seen to subdivide further and become more widely separated through the absorption of water, so that the fluidity and motility of the protoplasm is greatly increased. These changes continue until the critical point is reached, at which coagulation suddenly occurs, and the protoplasm goes into heat rigor, probably because of some chemical change in the protoplasm itself. So closely do these structural changes agree with those that occur in artificial colloids under the same conditions, that a description of one would apply equally well for the other. Indeed, it was this striking similarity between the results in the two cases, that led me to compare the reactions of colloids and the protoplasm of

[1] Greeley, *Amer. Jour. Physiol.*, 1901, VI., p. 201; Biol. Bull., 1902, III., p. 165; *ibid.*, 1903, V., p. 42.

various Protozoa to chemical and electrical stimuli to see what light these reactions might throw on the physical structure of protoplasm.

II. *Reactions of the Protoplasm of Paramœcium and other Protozoa to Chemical Changes.*

Paramœcia reared on cultures containing bread were used chiefly in these experiments, although other protozoa were utilized for purposes of comparison. The method employed in the experiments was as follows: The solutions whose action upon the protoplasm was to be tested were made up in the proper dilutions, and distributed among Minot watch glasses holding about 10 c.c. of the solution. Pure, concentrated cultures of the protozoa were obtained, and a drop or two added to each of the dishes containing the solutions. At short intervals the protozoa were examined to observe any structural changes in the protoplasm. A 1/12 inch oil immersion was used to make out the finer details.

Great care is needed to distinguish between chemical and osmotic effects. In order to obviate the possibility of modifying the protoplasm through the extraction of water, all the solutions were used in concentrations of a lower osmotic pressure than that of the protoplasm, roughly equal to a $m/40$–$m/50$ cane sugar solution. In some cases the dilutions were very great, and, inasmuch as distilled water or any solution of a lower osmotic pressure will liquefy the protoplasm through the absorption of water osmotically, there would be danger of confusing the osmotic and chemical effects of the solutions, were it not for the fact that osmotic and chemical liquefaction can be readily distinguished by the type of protoplasmic structure produced by each means.[1]

All the chemicals used fall into two classes:

1. Those that effect the protoplasm only through the osmotic pressure of the solution.

[1] Miss Towle, in investigations now in progress at the University of Chicago, has observed that paramœcia may live indefinitely in redistilled water. It is probable that in this case, as in others mentioned in this paper, the variations in the structural reactions are due to the differences in the chemical composition of the cultures in which the paramœcia were reared. But this question of the structural effect of distilled water requires further investigation.

2. Those that modify the structure of the protoplasm independently of the osmotic pressure. The first class includes the non-electrolytes used, the second, the electrolytes.

Non-electrolytes. — The non-electrolytes used, distilled water, cane sugar and urea, affect the protoplasm of paramœcia only through the osmotic pressure of the solution. There is no chemical effect. All those solutions hypotonic to protoplasm produce a liquefaction through the absorption of water, while isotonic solutions have no effect on the structure of the protoplasm, and hypertonic solutions coagulate the protoplasm through a withdrawal of water. These effects are shown in the following table:

TABLE I.

	m/5	m/10	m/40	m/160
Cane sugar,	Coagulation.	Coagulation.	No effect.	Liquefaction.
Urea,	"	"	"	"

An examination of these structural changes under a high magnification makes clear the fact that these non-electrolytes modify the structure of the protoplasm solely through a change in the amount of water in the fluid matrix which holds the more viscous elements in suspension. The size of the protoplasmic particles remains unaffected. In hypotonic solutions the protoplasmic particles are more widely separated by an increase in the amount of water in the fluid matrix. In hypertonic solutions they are brought closer together, or may fuse through a withdrawal of the surrounding liquid.

Electrolytes. — As far as their effect on the physical structure of the protoplasm of *Paramœcium* is concerned, all the electrolytes used fall into two classes: first, those that coagulate even in solutions whose osmotic pressure is far less than that of the protoplasm; second, those that liquefy the protoplasm in any dilution. Moreover, all the members of the first class agree as far as their electrical conditions are concerned. They all have a predominantly powerful cathion,[1] but resemble each other in no other particular. Likewise all the members of the second class agree in possessing a predominantly powerful anion. Thus we

[1] This "predominance" may be a function of the solution tension of the ions. See Mathews, *Amer. Jour. of Physiol.*, 1904, X., p. 290.

see that anions, or negatively charged ions, liquefy the protoplasm of *Paramœcium*, cathions, or positively charged ions, coagulate without any regard for the supposed chemical affinities of the electrolyte.

That we are not dealing with a specific chemical effect for each electrolyte is still further shown by the fact that the activity of each solution is roughly proportional to the valance of the predominant ion. Thus salts containing trivalent anions or cathions are effective in much greater dilutions than bivalent or univalent salts. All these facts are indicated in the accompanying table which gives a list of the electrolytes used, their effects on the physical structure of the protoplasm, and the greatest dilution at which they are effective. The maximal dilutions can be only approximate, as the action of identical solutions is not the same on paramœcia from different cultures, because no two are exactly alike in respect to chemical composition and osmotic pressure. Especially is this true of the liquefying electrolytes, for, as we have seen, in very dilute solutions it is frequently difficult to determine the point at which the specific liquefying action of the anion ends and the osmotic absorption of water commences. As has been already mentioned, there are structural differences between these two forms of liquefaction which enable us to distinguish between them with considerable accuracy, and the fact of chemical liquefaction can be easily demonstrated by using the liquefying substances in solutions about isotonic with protoplasm. Of course no such difficulty arises with the coagulating solutions, for at great dilutions the specific coagulating action of the cathion must overcome the tendency for the protoplasm to be liquefied through the entrance of water osmotically, and there is thus a well-defined point at which the coagulation of the protoplasm by the cathion ends and its osmotic liquefaction commences.

TABLE II.

Coagulating Solutions.	Liquefying Solutions.
$m/1{,}600$ HCl, $m/1{,}600$ HNO_3	$m/1{,}600$ NaOH, $m/1{,}600$ KOH
$m/2{,}400$ H_2SO_4	$m/1{,}600$ $Ba(OH)_2$, $m/1{,}600$ $Sr(OH)_2$
$m/40$ KCl	$m/40$ NaCl, $m/40$ NH_4Cl, $m/40$ $NaNO_3$
$m/640$ $MgCl_2$, $m/640$ $CaCl_2$, $m/640$ $Ca(NO_3)_2$, $m/640$ $BaCl_2$	$m/640$ Na_2SO_4, $m/640$ $(NH_4)_2C_2O_4$
$m/320$ $MgSO_4$	$m/2{,}400$ Na_3PO_4, $m/2{,}400$ $Na_3C_6H_5O_7$
$m/1{,}600$ Al_2Cl_6, $m/1{,}600$ Fe_2Cl_6	

It will be seen by an examination of the table that the activity of any of the salts, as is shown by the strength of solution required to modify the structure of the protoplasm of *Paramœcium*, varies directly with their valence. The acids and bases are much more powerful in their action than any of the salts of a similar valence, probably because of the known disproportionate kinetic energy of the hydrogen and hydroxyl ions.

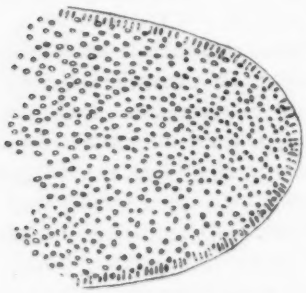

FIG. 1. Normal protoplasm of *Paramœcium*. $\frac{1}{12}$ in. oil immersion.

All the acids and salts found in the first column of the table, which agree in coagulating the protoplasm through the action of the predominant cathion, effect changes in the protoplasm so similar as to be practically indistinguishable even under a high magnification. The less active solutions, such as KCl and $MgSO_4$, do not produce quite so dense a coagulum as the others, and the reaction is considerably slower. But in all the bivalent and trivalent salts and the acids, a distinct clouding of the protoplasm can be observed within thirty minutes after the paramœcia have been immersed in the solution. This clouding of the protoplasm increases and is accompanied by a shrinking of the cell owing to a loss of water, until within a few hours, the whole cell is reduced to a subspherical mass of densely opaque protoplasm (see Fig. 2). The changes are identical with those produced by a lowering of the temperature.

FIG. 2. A *Paramœcium* in $m/320$ $MgCl_2$, showing a typical coagulation of the protoplasm.

An examination of the protoplasm with a one-twelfth inch oil-immersion lens reveals the fact that the clouding of the protoplasm is due to a separation of the two elements of the protoplasm, the cell sap and the protoplasmic granules. These two elements lose

their state of fine mixture or suspension, which is always characterisic of motile protoplasm, and the protoplasmic particles tend to fuse into a spongy coagulum or "gel" from which the cell sap continually escapes, until a complete separation of the two elements is brought about. This fusion of the protoplasmic particles may result in the formation of two varieties of coagulated structure. The fused particles may appear as spherical bodies of proteid material, unconnected but closely massed together in such a way as to form an exceedingly dense coagulum

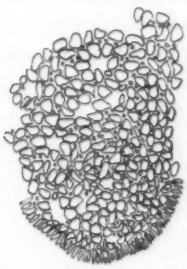

FIG. 3. Coagulated protoplasm of *Paramœcium* under $\frac{1}{12}$ oil-immersion. A dense coagulum formed after an exposure to *m*/320 $CaCl_2$ for twenty-four hours.

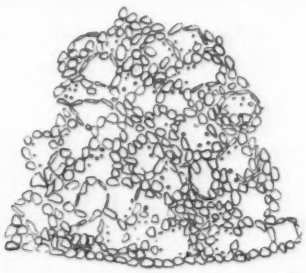

FIG. 4. Another type of coagulation viewed under the $\frac{1}{12}$ oil-immersion. The result of a ten hour residence in *m*/800 HCl, showing a trace of the reticular structure.

(see Fig. 3). Or the protoplasmic particles may fuse into fibrils or anastomosing threads which form an incomplete network, holding the cell sap in its interstices. The fibrils may eventually become thicker and transform the network into a relatively solid coagulum (Fig. 4). These last forms of coagulated structure are very similar to the network formations obtained by Hardy in organic colloids and various protoplasmic tissues by the action of fixing agents, and have been formerly supposed to be characteristic of living protoplasm. As in Hardy's experiments these fibrillar or reticular structures never appear in the living protoplasm of *Paramœcium*, but are solely an incidental result of the process of coagulation by chemical or other means.

The salts and bases in the second column of the table, which produce a common liquefaction of the protoplasm of *Paramœcium* through the action of a predominant anion, are effective like the

coagulating solutions, in dilutions which are roughly proportional to their valence.

The univalent salts have a comparatively weak effect upon the protoplasm, and relatively high concentrations are necessary before we can be sure that the liquefaction is due to chemical and not osmotic means. In solutions of the bases, bivalent and trivalent salts, however, the effects are unmistakable, and are exactly the reverse of those initiated by the coagulating solutions. Within a very few minutes after immersion in the solution liquefaction first becomes discernible as a clearing of the protoplasm. This process proceeds until the protoplasm loses its characteristically granular appearance, and becomes semi-transparent. At

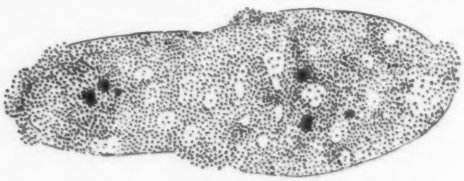

FIG. 5. A *Paramœcium* in *m*/320 Na_2SO_4, showirg a typical liquefaction of the protoplasm.

the same time the cell membrane becomes greatly swollen through the absorption of water, which frequently gives the protoplasm a vacuolated appearance, and gives rise to droplets which cling to the protoplasm underneath the cell wall. The result is an irregular watery mass of protoplasm from which the solid elements have, to a superficial examination, completely disappeared (see Fig. 5). In the solutions of trivalent salts these changes occur with such violence that the cell membrane is disrupted, and the disintegrated protoplasm becomes scattered throughout the solution. Thus the liquefying anion has the same effect upon the protoplasm as a slight increase in temperature.

If, as in the case of the process of coagulation, these microscopic changes be studied under a one-twelfth inch oil-immersion, it will be at once seen that a change in the physical state of the protoplasmic particles is responsible for these profound structural modifications. The particles apparently continue to divide as the process goes on, until their size becomes so small that they

are discernible only under high magnifications. At the same time a rapid absorption of water takes place, and the particles become widely separated in the now exceedingly fluid cell sap. We thus have a means of distinguishing between liquefaction by chemical and osmotic means. As has been described above, the central feature of the process of chemical liquefaction is a splitting up of the protoplasmic particles and consequent imbibition of water through this increase in the absorbing surface (see Fig. 6). In the process of osmotic liquefaction, however, the size of protoplasmic particles remains unchanged, and water enters the protoplasm solely because of the osmotic relations between the cell-sap and the surrounding liquid. These two processes can be distinguished microscopically as indicated even under a low magnification, for paramœcia that are liquefied osmotically never lose their granular appearance, while those liquefied chemically become markedly transparent.

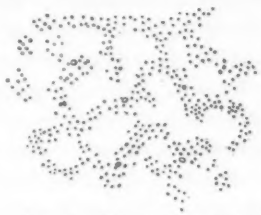

FIG. 6. Liquefied protoplasm of *Paramœcium* under $\frac{1}{12}$ oil-immersion. Formed by an exposure of two hours to $m/320$ Na_2SO_4.

That in these phenomena we are dealing also with variations in the surface tension relations of the protoplasm is apparent from the change of form which occurs during coagulation and liquefaction. The surface tension force is neutralized either by an increase in temperature or by giving all the protoplasmic particles a like charge which tends to make them mutually repellent, and thus introduces a disrupting force. Hence, if we destroy the electrical charge, we not only allow the protoplasmic particles to fuse, but we increase the surface tension. Thus, during coagulation, the cell tends to assume a spherical form which is characteristic of all resting cells. The opposite is true during liquefaction. The disrupting force is still further increased by the introduction of a charge of the same sign as that carried by the protoplasmic particles, and the cell assumes an irregular form. The most powerful anions used, the phosphate and citrate, bring about this disruption of the cell with almost explosive violence, so that the cell membrane bursts and the protoplasmic

particles are widely scattered. After a residence in these solutions of ten to fifteen minutes, no vestige of the paramœcia remains beyond the scattered protoplasmic granules.

The above experiments were repeated on *Vorticella*, *Stentor* and *Hydra*, and essentially the same structural changes were produced. The protoplasm of *Vorticella* reacts to electrolytes exactly as does the protoplasm of *Paramœcium*. In *Stentor* the differentiation between the two layers of protoplasm, endosarc and ectosarc, is more complete, and the less differentiated granular endosarc responds to the solutions in the typical manner, while the clear striated ectosarc remains practically unaffected. Thus the process of coagulation results in the formation of a cell whose endosarc is shrunken into a dense, spherical mass within the ectosarc, which retains its original form and size. During liquefaction the endosarc becomes wholly transparent, and we have produced an apparently empty cell which is bounded by the unchanged ectosarc as before. *Hydra* reacts in the same manner, the endoderm responding to the solutions as did the endosarc of *Stentor;* and we have produced animals of the original size and shape, but with either a coagulated or liquefied interior. It appears from these experiments that the physical conditions already described are applicable to protoplasm only in its primitive granular condition. The differentiation into such simple structures as the ectosarc of *Stentor*, or the ectoderm of *Hydra* has changed to some extent its elementary physical state. The high degree of viscosity of such protoplasm suggests that it is normally much nearer the "gel" phase.

Like all the typical physical modifications of protoplasm, these changes of structure are reversible, if they are stopped at the proper time. Thus coagulated protoplasm may be again liquefied by the action of a powerful anion, or liquefied protoplasm may be coagulated by a cathion. This fact seems to show with special force that the physical state of the protoplasm at any moment is the result of a definite reaction to the chemical conditions of the environment.

The whole behavior of protoplasm under the action of dilute solutions of electrolytes suggests the conclusion, that, as far as its chemical and physical reactions are concerned, it is a colloidal

solution whose particles carry a definite electrical charge. The colloidal particles in an artificial colloidal solution are apparently identical, as far as their physical reactions are concerned, with the protoplasmic or proteid particles held in the cell sap of protoplasm; at least they both react to thermal and chemical changes in the same way. The reaction to chemicals further demonstrates the fact, that, like the colloidal particles, these protoplasmic elements carry a uniform electrical charge under normal conditions; for by no other assumption can we explain the facts that salts, acids and bases modify the structure of protoplasm only by virtue of their electrical properties, and that non-electrolytes have no effect whatever beyond that of the osmotic pressure of the solution. Indeed the action is not, properly speaking, a chemical one at all, but an electrical one. In *Paramœcium* the protoplasmic particles are apparently negatively charged, and the mutual repulsion of these similarly charged particles, keeps them in a state of fine suspension. Under these conditions the protoplasm exists in a "sol" or liquid phase. Predominant cathions neutralize this negative charge; the repelling force is removed, and the particles fuse, forming a coagulum. A predominant anion further increases the disrupting force, and a state of still greater fluidity or liquefaction results.

A still closer comparison between artificial colloids and the protoplasm of *Paramœcium* is possible. It will be remembered that Hardy succeeded in reversing the charge carried by the colloidal particles by changing the chemical conditions of the solution. Thus the addition to the solution of a small amount of a base gave an alkali-modified colloid whose particles were negatively charged. Such a solution was coagulated only by cathions. Likewise the addition of a small amount of acid gave an acid-modified solution, whose particles were positively charged; this solution was coagulated by anions. How this change is effected by acids and alkalis we cannot say, but certain it is that the electrical nature of the colloid is determined by certain ions present in its fluid matrix.

The reactions of the protoplasm of paramœcia to electrolytes described above, occurred in organisms which had been reared in a normal, alkaline culture. It was found that when the culture

medium became acid through the fermentation of bread, the structural reactions of the protoplasm to electrolytes was changed. And they were changed in such a way as to suggest that there had been a reversal of the electrical charge carried by the protoplasmic particles, or in other words, that there had been formed an acid-modified protoplasm. Thus the protoplasm of paramœcia from an acid culture was not coagulated by cathions and liquefied by anions, as was the case without exception with paramœcia from an alkaline culture, but the reactions were as follows: The first change produced by the neutralization of the normal alkalinity of the culture was an irregularity in the structural reactions. Instead of all the organisms being coagulated by cathions and liquefied by anions, some were coagulated, and some were liquefied in each solution, apparently indicating a condition in which the protoplasm of some of the paramœcia had been modified by the acid, the rest being still unaffected. As the acidity of the solution increased, the number that were coagulated by anions and liquefied by cathions correspondingly increased until, in a few instances, a complete reversal of the structural reaction to electrolytes was produced. While the reversal was more often not complete, in all cases it occurred in a varying proportion of the paramœcia from acid cultures, while the remainder were generally rendered indifferent to the solutions which formerly coagulated or liquefied them. The complete reversal occurred only in the salt solutions. The acids always coagulated the protoplasm regardless of the character of the culture, and to alkalis, the paramœcia from acid cultures were only rendered indifferent. All these results indicate that the particles of the acid-modified protoplasm have become positively charged like the particles in Hardy's acid-modified colloids.

The view that this change in the structural reactions of paramœcia is due to a modification of the protoplasm by acids is rendered more probable if we follow the reactions of paramœcia from day to day, which are taken from a culture which is gradually becoming acid by the fermentation of bread. In such a case we can easily see the gradual fading out of the normal reaction to electrolytes, and the assumption of the one peculiar to acid-modified protoplasm. This change in structural reaction always

accompanies the neutralization of the alkalinity of the culture. The complete reversal in the sign of the charge carried by the protoplasmic particles is apparently too fundamental a change to occur without killing the protoplasm except under the most perfect conditions. While much more work must be done on this point, we can assuredly say that the normal electrical charge carried by the protoplasm of *Paramœcium* bears a very close relation to certain chemical conditions of the environment, of which the alkalinity of the surrounding medium may be taken as one of the most important. These results are especially significant in the light of the behavior of paramœcia from various cultures toward different forms of stimuli, as we shall see when we discuss this subject.

III. *Reactions of the Protoplasm of Paramœcium to the Electrical Current.*

The above conclusions relating to the ultimate physical structure of protoplasm and the electrical conditions underlying it, are further borne out by a study of the effects produced on the structure of the protoplasm by the constant current. It has long been known that the constant current has a profound polar effect on the protoplasm of various protozoa.

Thus Kühne, Verworn[1] and others have shown that when a protozoan is exposed to the action of a weak current, a contraction occurs on the anodal side of the cell, and a relaxation on the cathodal side. This phenomenon was first described for *Actinosphærium*, a large heliozoan with many radiating pseudopodia. Very soon after exposure to the current the pseudopodia on the anodal side become contracted into irregular shapes, and are finally completely withdrawn into the cell, while those on the cathodal side remain fully extended. *Amœba* is still more sensitive to the current. The whole cell contracts on the anodal side while pseudopodia are rapidly thrown out toward the cathode so that the animal moves in this direction. The same general changes have been observed in *Paramœcium*[2] and many other protozoa. If the organisms are exposed to the current for a

[1] Verworn, *Arch. f. d. ges. Physiol.*, 1889, XLV., p. 1.
[2] Pearl, *Amer. Jour. of Physiol.*, 1900, IV., p. 96.

longer time, more pronounced structural changes ensue. The contraction on the anodal side continues until the protoplasm at this point can be seen to disintegrate, forming dense granular masses, while on the opposite side the protoplasm becomes even more clear and transparent than it was at first, frequently flowing out over this portion of the cell in irregular liquid masses. The observations have been repeated in a large number of forms so that the facts of definite polar modifications in the structure of the protoplasm during the passage of a weak constant current seem to be very well established. At the same time it has been shown in many forms that there is a movement of the protoplasmic particles away from the cathodal side where liquefaction of the protoplasm is taking place toward the anodal region of the cell where the disintegrating or "etching" effects are shown.[1]

I have repeated these experiments and have furthermore shown that when paramœcia are isolated in a weak gelatine solution or held in a fine mesh-work of cotton during the passage of the current so that they are unable to move freely from pole to pole, a slightly different structural reaction to the current is obtained after the current has been passed for from three to five hours. In this case all those paramœcia which are held in the region of the anode are modified as was the anodal side of the cell in the preceding experiment, *i. e.*, the whole cell contracts into a dense, opaque mass of protoplasm. Likewise the paramœcia held about the cathode are modified like the cathodal side of the cell in the former experiment, *i. e.*, the cell contents are liquefied and there is formed a large transparent cell of fluid protoplasm which ultimately bursts because of the increased pressure on the cell wall. Thus we have the same structural changes occurring either in the whole cell immediately about the anode, or in the anodal side of any of the cells exposed to the current; opposite changes occurring in the cells about the cathode or on the cathodal side of all the cells in the preparation.

A microscopical examination shows that these changes are

[1] Wallengren (*Zeitschr. f. allg. Physiol.*, 1903, III., p. 22) states that in the Rhizopoda only can the movement of the protoplasmic particles toward the anode be demonstrated. In the Infusoria the constant current does not affect the normal streaming of the protoplasm.

identical with the structural changes produced by the action of electrolytes upon the protoplasm. About the anode and on the anodal side of the cells the protoplasm is coagulated as by the use of cathions in weak solutions; about the cathode and on the cathodal side of the cells, the protoplasm is liquefied as by the use of anions in weak solutions. Not only are these structural effects the same, but they can be shown to be produced by the same means in each case, *i. e.*, by electrically charged ions, which are present in very dilute solutions of electrolytes, as we have shown, and which serve to carry the current from pole to pole when it is passed through such weak salt solution as the culture media of paramœcia which were used in the experiment.

It was formerly supposed that these polar effects were due to the formation of acids on the anodal side of the cell and alkalis on the cathodal side, but we have no satisfactory evidence for such internal polar changes. During the passage of the current, the anions prevail about the cathode, and are constantly diffusing toward the anode. During this passage they are continually impinging on the cathodal side of the paramœcia, hence a liquefaction of the protoplasm takes place at these places. Cathions prevail about the anode, and continually impinge on the anodal side of the cells in their diffusion toward the cathode so that coagulation occurs at these points. In either case the structural changes are produced by virtue of the electrical charge which the ions carry, and not by any specific chemical effect of the ions or molecules. We thus see that the chemical and electrical means of modifying the protoplasmic structure are identical.

It will be remembered that these changes in the structure of the protoplasm produced by solutions of electrolytes or the electrical current are the same as those which are brought about in organic colloids by the same means. The alkali-modified colloids in Hardy's experiments were coagulated by cathions in solution or at the anode during the passage of the current. They were liquefied by anions or at the cathodes. It is interesting to note also that in the protozoa a movement of the protoplasmic particles within the cell toward the anode has been observed, which corresponds exactly with the movement in the same direction among the colloidal particles of an alkali-modified colloid. Like

the colloidal particles, they always move toward the point at which coagulation occurs.

All these experiments seem to show that the structural changes produced in protoplasm by thermal, osmotic, chemical or electrical changes are the same, because all of these variations in the external conditions act upon protoplasm only by altering the physical state of its solid elements. Thus in the case of *Paramœcium*, at least, the structure of the protoplasm is seen to be not fixed and uniform, but to depend directly on certain external conditions and to vary with their variations. The best expression of this behavior of protoplasm is found in the laws of the reaction of colloidal solutions to external conditions.

Effect of these Structural Modifications on the Vital Properties of the Protoplasm.

Having determined the changes produced in the structure of the protoplasm by various chemical agencies, it remained to ascertain how these structural changes modify the vital properties of protoplasm. How far may a particular state of protoplasmic activity be correlated with a given physical condition of protoplasmic structure? Or does the reaction of an organism as a whole to an external stimulus depend in any measure upon the effect that stimulus may have on the structure of the protoplasm?

I. Growth and Cell Division.

The rate of cell division may be taken as the best indication of the general protoplasmic activity among the Protozoa, after the method adopted by Calkins[1] in his work on the "Life Cycle of Paramœcium." A quickened rate of cell division means an increase in the metabolic activities of the cell. A condition of slow metabolism is indicated by the cessation of cell division and the transformation of the motile cell into a spore or cyst or other resting stage. It has been already shown, in a paper[2] on the reactions of various protozoa to variations in the temperature, that precisely those temperature conditions, which liquefy the protoplasm, stimulate cell division, and those temperatures which coagulate

[1] Calkins, *Arch. f. Entwickelungsmech.*, 1902, XV., p. 139.
[2] Greeley, *Amer. Jour. Physiol.*, 1902, VI., p. 122.

the protoplasm inhibit it. Thus the rate of cell division increases steadily with a slight elevation of temperature above the normal until the critical coagulating point is reached. A lowering of the temperature progressively decreases the rate of cell division, until the point is reached at which it ceases altogether, and the protoplasm goes into a resting condition.

The same relation between the rate of cell division and the physical state of the protoplasm is found to hold good also as a result of the reactions of the protoplasm of *Paramœcium* to solutions of electrolytes.

With paramœcia from alkaline cultures, anions or liquefying agents stimulate cell division, cathions and coagulating agents inhibit it. Thus I have frequently observed in my experiments that when the liquefying solution is too weak seriously to modify the structure of the protoplasm, it will, however, greatly increase the motility of the protoplasm and the rate of cell division. Since the size of the paramœcia remains uniform, it follows that this must indicate also increased growth and general metabolic activity. In the coagulating solutions, on the contrary, there are produced spherical resting cells that greatly resemble spores. This antagonism between these two classes of solutions is still more clearly shown by the fact that the anions greatly accelerate the germination of spores, or the passage from a resting into a motile condition. In these solutions the spherical form of the spore is soon lost through a neutralization of the surface tension. This has been shown in the case of some of the monads and fresh-water algæ.

R. S. Lillie[1] has shown that a decrease in the surface tension must accompany cell division, and that this is accomplished in the case of certain marine animals by the electrolytes present in the sea water, for if these electrolytes be withdrawn, cell division not only stops, but a partial fusion of the already formed blastomeres occurs. It appears that the surface tension relations are very important in all these protoplasmic reactions to external conditions. Protoplasmic movement, cell division and growth all occur in opposition to the surface tension force.[2] Consequently any external condition which neutralizes the surface ten-

[1] Lillie, BIOL. BULL., 1903, IV., p. 164.

[2] See Spaulding, BIOL. BULL., 1904, VI., p. 97.

sion accelerates these expressions of the general activity of the protoplasm. These conditions are those which bring about a liquefaction of the protoplasm, so that the protoplasmic activity is seen to vary directly with the amount of water the protoplasm contains. Conversely the assumption of a quiescent spherical resting stage in various protozoa is the result of an increase in surface tension, and is formed by those conditions which cause a coagulation of the protoplasm and a loss of water. Thus a slight increase in the temperature is seen to have the same effect on these simple protoplasmic properties of *Paramœcium* as anions ; a lowering of the temperature acts like cathions ; since each set of conditions produces the same structural effects.

II. *The Tropisms.*

We have at the present time an enormous amount of information concerning the reactions of organisms to external stimuli, but we know almost nothing of the physical or chemical effects of these attractive or repellent agents on the protoplasm of the organism, and consequently we are not able to offer any satisfactory explanation of the mechanism of the tropic response. In the following experiments I have studied the reactions of paramœcia to thermal, electrical and chemical stimuli, and have attempted to show that the reaction of *Paramœcium* to each stimulus depends on certain structural changes in the protoplasm, which are a result of the stimulating action.

1. *Thermotaxis.* — The reactions of paramœcia to variations in temperature are exceedingly definite. It has been stated by many observers that they are positive to temperatures between approximately 23° and 27° C., and are negative to all others. This reaction is beautifully demonstrated by placing the paramœcia in a long, narrow dish which is heated at one end and cooled at the other. It has been already shown that those temperatures to which *Paramœcium* is positive constitute exactly those thermal conditions which bring about a liquefaction of the protoplasm and a reduction in the surface tension. Those temperatures to which *Paramœcium* are negative, coagulate the protoplasm. We thus see that, in the case of thermotaxis, attraction is accompanied by a liquefaction of the protoplasm, repulsion by coagulation. The

extreme delicacy of the adjustment between the physical structure of the protoplasm and the external conditions has hardly been recognized. For example, the smallest perceptible elevation of temperature above the normal results in a decided increase in the fluidity of the protoplasm, and it is probable that this structural change explains the extreme sensitiveness of the paramœcia, judged by their thermotropic reactions, to these same variations in the temperature.

2. *Galvanotaxis.* — It has been well known that paramœcia normally react to the electrical current in a vigorous and definite manner by orienting themselves with their anterior end toward the cathode, and swimming rapidly in this direction, so that eventually a dense gathering of the organisms occurs about the negative electrode. In other words they collect at that point in the electrical field where the conditions are such as to induce a liquefaction of the protoplasm. After a weak current has been passed through the preparation for from thirty minutes to one hour, it will be seen that the dense gathering at the cathode begins to break up and a reverse movement toward the anode sets in. The number of paramœcia that exhibit this reverse movement varies with the conditions of the organisms at the time of the experiments, as will appear later; but with paramœcia from alkaline cultures only a small proportion of the entire number will be seen to swim toward the anode at any one time. At first the paramœcia swim only a short distance toward the anode and then immediately dart back to the cathode, but the length of the reverse reaction increases until a few reach the anodal end of the dish. Having arrived at the anode, they immediately swim back to the cathode again, and no gathering occurs at the anode except in rare cases after the current has been passed for about two hours. The paramœcia normally keep the anterior end pointed always toward the cathode, so that they swim backwards toward the anode. But this is not always the case.

After a large number of experiments with paramœcia under various conditions, I find that the relations between this initial and secondary reaction to the current may be greatly modified. With paramœcia from alkaline cultures, the secondary reaction begins only after the current has been passed for thirty minutes

or more and is at first of an exceedingly transitory nature. But with paramœcia that have been reared in a culture that has been made slightly acid by the fermentation of starch, and hence are in an acid-modified condition as before described, the secondary reaction may begin as soon as the paramœcia reach the cathode. In these cases no gathering occurs at the cathode but each *Paramœcium* immediately reverses the stroke of the cilia and swims back to the anode. The process is repeated at that point, so that we have for a time no collection at either pole but a continuous line of paramœcia swimming in either direction until eventually they come to rest in about equal numbers at each end of the preparation. Under the most favorable acid conditions a number of paramœcia, varying from one to fifty per cent. of the whole number, exhibit an initial reaction toward the anode and a secondary reaction toward the cathode, while the remainder react in the manner described above.

That this immediate reversal of the normal reaction and the initial response toward the anode are due to the acidity of the culture medium may be shown by the following experiments. If 5 c.c., of a neutral[1] culture of paramœcia be isolated and tested to the current, it will be found that they all exhibit the characteristic response toward the cathode and form a dense gathering at that point. If now, however, from two to four drops of an $m/10$ solution of hydrochloric or other acid be added to the culture, and, after standing for thirty minutes, the reaction to the current be again tested, it will be found that either an initial or an immediate secondary reaction toward the anode has set in. Also the addition of a small amount of acid to an already acid culture invariably strengthens the anodal response of paramœcia. Likewise in every case in which I have tried it, the neutralization of the acid with NaOH, or the addition of the solution of a salt with a trivalent anion like Na_3PO_4 or $Na_3C_6H_5O_7$ entirely destroys both the initial and the secondary response toward the anode, and leaves only the characteristic gathering at the cathode.[2]

[1] It is necessary to use paramœcia from an approximately neutral culture for this experiment. The normal reaction toward the cathode is too firmly fixed in paramœcia from a strongly alkaline culture to be reversed.

[2] More striking results have been obtained with *Volvox*. After an exposure of half an hour or more to a slightly acid medium, practically every organism completely reverses its response, so that a dense gathering is formed about the anode.

In several instances, after a prolonged exposure to a medium which had been acidulated by the addition of hydrochloric or acetic acid, the paramœcia exhibit still another form of reaction. In these cases the paramœcia orient themselves transversely or slightly obliquely to the direction of the current, and swim very slowly from side to side of the preparation. At the same time, however, the organisms appear to drift passively toward the anode. I observed this form of reaction only about half a dozen times, but on each occasion the reaction was exceedingly well marked. A reversal of the current caused an instant reversal, of both the direction of the swimming and the passive drifting of the organisms. Notwithstanding the peculiar orientation of the paramœcia, they all tended to form a gathering about the anode under these acid conditions.

It has been observed by Loeb, Jennings and others that the addition of other substances, like NaCl, to the solution containing the paramœcia will cause a reverse movement toward the anode. I investigated this question and found that the addition of almost any salt in sufficient quantities to extract water from the protoplasm will cause a more or less complete reversal. A large number of salts, of both positive and negative electrical conditions, were used, and the effect was seen to be purely osmotic in character, except in the case of the salts with trivalent anions or cathions. The former, as the phosphates and citrates, act like the hydrates in very weak solutions and completely destroy all traces of a response toward the anode. The latter, as Al_2Cl_6, produces an almost instant coagulation of the protoplasm and hence bring about the same effect as the extraction of water osmotically. It has been already stated that a lowering of the temperature coagulates the protoplasm, and it is interesting to note, that a partial reversion of the electrical response may be produced by this means also. After the paramœcia have been exposed to a temperature of 2° to 3° C., for one hour or more, the normal response is entirely lost, and a slight movement toward the anode can be detected.

Our ignorance of the precise nature of the ciliary response is too great to allow even an attempt at an explanation of the mechanism of this response to the electrical current. We will

first have to obtain a satisfactory explanation of the rhythmical contraction of the ectosarc which controls the cilia. It is certain that the surface tension relations of the muscular elements play an important rôle in this process. In *Amœba* the problem is very simple. The protoplasm contracts on the anodal side because of the neutralization of the charge carried by the protoplasmic particles and consequent increase in surface tension. Pseudopodia are thrown out on the cathodal side, and movement occurs in this direction because of the decrease in surface tension at this point. But for paramœcia it suffices at present to show that the sense of the response is not a fixed attribute of the organism which has been acquired by natural selection, but that it is a purely physical response to an external stimulus, and varies directly with the conditions under which it occurs. The ultimate determining factor of the response to the electrical current must be the electrical conditions of the protoplasm itself. In paramœcia from a normal alkaline culture the protoplasmic particles appear to be negatively charged. These paramœcia collect about the cathode where liquefaction of the protoplasm occurs. But with precisely the same conditions, under which liquefaction is produced not by anions but by cathions, *i. e.*, an acid culture medium, we find that the characteristic gathering about the cathode does not occur, but the paramœcia tend to move toward the anode where the protoplasm would now become liquefied. The only explanation of this phenomenon that presents itself is the assumption, that in an acid medium the protoplasmic particles become positively charged in a portion of the organisms (for the reaction is never completely reversed). The partial reversal of the reaction by osmotic means is also due to an alteration in the electrical conditions of the protoplasm, as has been shown.

Hardy's acid and alkali-modified colloids reacted also in an opposite manner to the electrical current because in the acid solution the particles were positively, and in the alkaline solution negatively charged; and while the explanation is not so simple in the case of *Paramœcium*, because movement is effected by a complex motor apparatus, still the sense of the reaction must be ultimately due to the same cause in both cases, *i. e.*, the charge carried by the colloidal or protoplasmic particles. Moreover,

Hardy observed the same secondary reversed movement of the colloidal particles as has been observed in the reaction of *Paramœcium*. All these facts make very evident the similarity which exists between the electrical conditions in the two solutions. Lillie[1] has observed a similar relation between the response to the electric current and the chemical condition of the protoplasm whether acid or alkaline, in his experiments upon nuclear and cytoplasmic structures cited at the beginning of the paper. He shows, that, when exposed to the electric current, nuclear structures, which contain a large amount of nucleic acid, move toward the anode, while cells very rich in cytoplasm, which is basic in reaction, move toward the cathode.

3. *Chemotaxis.* — A large number of important contributions have been made during the last few years to our knowledge of the reaction of protozoa to chemical stimuli. Most notable have been the remarkable series of investigations carried on by Jennings, who has given us not only a complete account of the sense of the reaction of many protozoa to a wide range of chemical substances, but also an accurate description of the method of the reaction in each case. My own results agree with those of Jennings in all essential points. I confirm his account of the "motor reaction" of *Paramœcium* when under a chemical stimulus. It was not my purpose to repeat any of Jennings' experiments, but only to ascertain the chemotropic reactions of *Paramœcium* under various conditions, to see if they could be modified by external influences as was the galvanotropic response. The only respect in which my conclusions depart from Jennings' is that my experiments seem to show that the chemotropic reactions of *Paramœcium* which he describes are not of universal occurrence, but limited to paramœcia which have been reared under definite chemical surroundings. In other words, the sense of the chemotropic reaction, like the galvanotropic, depends upon certain chemical conditions of the environment.

Jennings found that paramœcia were in general positive to weak acids (*i. e.*, formed a gathering within the drop) and negative to weak alkalis. He also observed that they reacted in a constant manner to a large number of salts. In my own experi-

[1] Lillie, *loc. cit.*

ments, I find, that only those paramœcia which have been reared in a slightly acid culture medium are positive to acids, and that paramœcia from clear alkaline cultures are negative to acids and positive to alkalis. It appears also that salts with trivalent cathions act like acids, and salts with trivalent anions act like alkalis. No definite conclusions could be drawn from the action of the univalent and bivalent salt solutions. For the purpose of the experiments, we are most vitally concerned with the reaction of those solutions which carry the heaviest positive or negative charges of electricity.

The greatest caution is needed in determining the acidity or alkalinity of the culture medium. A carefully prepared litmus solution is the best indicator. Phenol-thalein may also be used for the detection of small amounts of alkalis. Paramœcia freshly reared in a culture whose alkalinity has been determined in this way invariably react to the solutions used as follows: To $m/200$ HCl, HNO_3, H_2SO_4, and acetic acid; $m/800$ Al_2Cl_6 and Fe_2Cl_6, they are negative. To $m/200$ NaOH, KOH, $Ba(OH)_2$ and $Sr(OH)_2$; $m/480$ $Na_3C_6H_5O_7$ and Na_3PO_4, they are positive. The positive reaction is shown by the organism swimming into the drop, and then giving the motor response, described by Jennings, when they come in contact with the outside water, so that a gathering is formed within the drop. The solutions to which the paramœcia are negative provoke the motor response when the organisms first come in contact with the outer edge of the drop, with the result that the solution is left empty. In some cases the paramœcia appear to be entirely indifferent to a solution, and swim in and out in an undisturbed manner. Such a reaction will also be classed as negative.

If the paramœcia from the same alkaline culture be tested from day to day as the alkalinity is being gradually neutralized by the formation of acid during the fermentation of bread, or by the addition of free acid to the culture, it will be seen that the response to these solutions slowly changes, until finally the chemotropic reaction is completely reversed, and now in the acid medium the paramœcia are positive to acids and salts with a trivalent cathion, and negative to alkalis and salts with a trivalent anion. Likewise, if the culture be again made gradually alka-

line, the first form of reaction, characteristic of alkaline cultures, returns, so that it becomes evident that the sense of the chemotropic reaction depends directly on certain chemical conditions of the surrounding medium. The transformation from the first form of reaction to the second, and *vice versa*, is a very gradual one, so that it is not immediately effected by the chemical change in the surrounding medium, and a considerable time may elapse between the neutralization of the alkalinity of the culture, for example, and the loss of the positive response to alkalis; but eventually the reaction occurs as has been described. Thus paramœcia are seen to seek out those chemical conditions which bring about a liquefaction of the protoplasm. The sense of this response also is apparently determined by the electrical condition of the protoplasm.

An interpretation of the mechanism of this response to electrolytes is as impossible as it was in the case of the reaction to the electric current. But, since in each case the paramœcia collect under the same electrical conditions, both responses must be ultimately due to the same reaction of the contractile layer of the protoplasm to electrically charged ions, and this reaction must consist largely in the effect which the electrically charged ions have upon the surface tension of the contractile elements of the protoplasm. Experiments upon *Amœba* bear out this hypothesis. Cathions always produce a contraction of the protoplasm, while anions produce a relaxation or the extension of pseudopodia, because the former increase the surface tension of the protoplasm while the latter neutralize it. Thus *Amœba*, like *Paramœcium*, is positive to predominately negative solutions, but in the one case the response is accomplished by the immediate effect which the anions have upon the surface tension of the protoplasm, while in the other case it is brought about through the agency of a complex motor apparatus.

Conclusions.

We see that precisely those chemical changes in the surrounding medium, which modify the structural reactions of the protoplasm of *Paramœcium* to solutions of electrolytes, modify also the reactions of the organism to electrical and chemical stimuli.

The protoplasm of paramœcia from an alkaline culture is liquefied by temperatures between 23° C. and 30° C., by anions, and at the cathode during the passage of the constant current. The paramœcia also react positively to all these chemical and physical conditions. The protoplasm of the same paramœcia is coagulated by temperatures below 20° C. and above 30° C., by cathions, and at the anode during the passage of the current. The organisms are negative to all these conditions. The structural changes produced by electrolytes are partially reversed in paramœcia from a slightly acid culture, and the reactions of the organisms are also partially reversed to the electric current, completely so to solutions of electrolytes. In every case the reaction of a *Paramœcium* to an external stimulus leads it to remain under those conditions which liquefy the protoplasm. Attraction is accompanied by liquefaction, repulsion by coagulation. As far as the physical structure of the protoplasm is concerned, the conclusion from these facts seems to be that the protoplasmic particles are physically identical with colloidal particles. Hence the protoplasm of *Paramœcium* is essentially a colloidal solution whose particles carry a definite charge of electricity. The sign of this charge appears to depend on certain external chemical conditions, of which the alkalinity of the surrounding medium may be taken as one of the most important. The sign of this charge is seen to determine not only the structural modifications of the protoplasm, but also the reactions of the paramœcia to chemical and electrical stimuli. This conception of the physical structure of protoplasm is also used to explain the effect of external conditions on the processes of cell division, growth and movement through the operation of the laws of surface tension.

ZOÖLOGICAL LABORATORY,
WASHINGTON UNIVERSITY.
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EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF ORGANS IN THE EMBRYO OF THE FOWL (GALLUS DOMESTICUS).

FRANK R. LILLIE.

II. The Development of Defective Embryos, and the Power of Regeneration.[1]

Born has shown that young embryos of the frog possess immense power of healing smoothly cut wounds, and that the vitality of even small isolated parts is remarkable; pieces of the head or of the tail, for instance, may continue to grow and develop in spite of the complete absence of the heart, blood and blood vessels, and nervous system, for a period of about three weeks, or until the yolk contained in the cells is fully consumed. "The development of each organ progresses as far as the cut surface as well as in the normal larva, no matter what the position of the cut surface may be; the absence of the heart or the brain does not affect the subsequent processes of growth and differentiation in any marked way."

These conclusions are of great importance for the physiology of development. So far as I know, similar experiments have not been performed on the embryos of *Amniota*, and the following account may serve to fill up the gap in part. Striking differences appear between the embryo of the fowl and of the frog in regard to the vitality of defective embryos, or of parts of embryos: The chick embryo dies very rapidly after destruction of the heart, for the embryonic tissues are dependent to an extreme degree upon the circulation; it therefore follows that small parts of an embryo cannot undergo differentiation as in the frog. On the other hand, the growth of the extra-embryonic blastoderm is independent of blood supply, and may continue in eggs in which there is no embryo owing to destruction of the heart, until nearly the entire yolk is covered. This difference between the embryo of the chick and its own extra-embryonic blastoderm or the embryo of

[1] The first part appeared in Biol. Bull., 1903, Vol. V., No. 2: "Experiments on the Amnion and the Production of Anamniote Embryos."

the frog, may be explained by the simple consideration that the cells of the embryonic tissues proper in the chick are devoid of yolk or other nutriment, and hence are dependent for their subsequent growth and differentiation upon the circulation ; whereas the cells of the frog embryo are loaded with food in the form of yolk ; and the extra-embryonic blastoderm of the chick is a digestive organ lying on an immense reservoir of food.

The necessity of circulation for all normal development of the chick embryo beyond the stage of about 33 hours (12–14 somites) at once limits the range of defective embryos capable of development to those possessing a heart and vitelline circulation. Another limitation arises from the extreme sensitiveness of the embryo to removal of parts of the brain ; although I have made over seventy experiments on the brain, none of the embryos, in which the injury extended back of the optic stalks, has developed for more than about forty-eight hours after the operation. (The operations on the head form a class in themselves and will be discussed in a separate paper.) The reason for the large number of fatalities in operations in this region is probably not due to any trophic function of the nervous system, at least in stages younger than 72 hours, but either to direct injury to the anterior end of the heart, or to malformations of the amnion consequent on the operation. On the other hand, embryos may survive the destruction of a considerable portion of the posterior end, and develop normally for several days as least. All the defective embryos to be described resulted from operations of this kind, performed, with one exception, on embryos in which the tail-bud is just forming after 50–60 hours' incubation.

A larger or smaller part of the posterior end was destroyed by cauterization. The embryos might bear complete destruction of the posterior end up to the vitelline arteries, provided these were not injured, without any apparent detriment or hindrance to the development of the uninjured parts. If the vitelline arteries were destroyed, the embryo never survived. Fig. 1 gives a view of an embryo of about the age of those used for operations. In this case 29 somites are formed ; the number was certainly not over 30 at the hour of operation in any case. The somites are continued backward by the undivided segmental plate represent-

ing a considerable number of potential somites. As will be shown later, somites 26 to 32 are the ones that normally form the musculature of the leg. Thus the undivided mesoblast at this stage includes a large part of the trunk. The vitelline arteries at this time leave the embryo opposite to the twenty-first or twenty-second somites; this position appears to be very constant. Thus the theoretical limit of the experiments is from the hind end to about the twenty-second somite, because

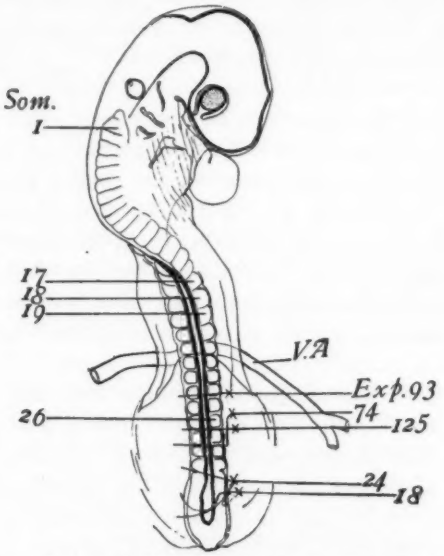


FIG. 1. Camera drawing of chick embryo with 29 somites; operation diagram. For explanation see text.

the embryo cannot live if the vitelline arteries are destroyed. The rudiment of the allantois lies beneath the unsegmented mesoblast beginning, approximately, a short distance back of the thirtieth somite.

Fig. 1 serves at the same time as a diagram of the operations. At the time of each operation a sketch was made of the embryo, and the part destroyed was indicated by shading the posterior end correspondingly. This sketch, naturally, did not show somites, so the present operation diagram is constructed from data deter-

mined after the operation. It agrees, however, fairly well with the original operation diagrams which were simply rough estimates of the amount destroyed by the operation. It should be distinctly understood that the figure is a result of study of the anatomy of the defective embryos. In further explanation it should be added, that the numbers to the right are the numbers of the experiments; those to the left of the somites. The lines leading to the numbers indicating the experiments are drawn across the body to indicate, that, in the experiment in question, all back of the line was destroyed; the cross on each line marks the junction of reference and operation lines.

A word concerning the enumeration of the somites; the somite numbered 1 possesses a short anterior process, that is probably an independent somite. It is, however, so inconspicuous in many embryos that it seemed better for the present purpose not to enumerate it. Somites 17, 18 and 19 are especially marked because they are the wing-somites, *i. e.*, the somites that will form the major portion of the bone and muscle of the wing. The twenty-sixth is the first leg somite. By this reckoning, there are three cephalic somites, or, reckoning in the incomplete one, four.

Referring to Fig. 1 again, it will be seen that the organs destroyed in such operations are: (1) The hind end of the neural tube; (2) the hind end of the notochord; (3) the mesoblastic segmental plate and often certain of the posterior mesoblastic somites; (4) the hind gut including the rudiment of the allantois; (5) the hind end of the Wolffian body and ducts.

I may say at once that no true regeneration of these structures takes place. (For discussion of this, see p. 50.) Therefore the problems of interest became narrowed down to the differentiation of the uninjured parts, and my attention has been particularly drawn to the behavior of the mesoblastic somites. In the somites we have an originally homonymous series of structures, the segmentation of which becomes strikingly heteronymous as development proceeds, some entering into the head, others the neck, others the wing, the thorax, abdomen, leg and tail. Definite somites, *i. e.*, somites in a definite numerical position in the series, form the skeleton and muscles of each of these regions. It would

be interesting to determine whether or not somites had the same rôle in defective as in normal embryos.

The numerical value of the somites in the chick seems to be normally as follows:

96-hour Chick.		Eight-day Chick.	
1, 2, 3,	Cephalic, hypoglossus region.[1]		
4 to 16,	Prebrachial, trunk.	1 to 13,	prebrachial.
17, 18, 19,	Brachial.	14, 15, 16,	brachial.
20 to 25,	Between wing and leg.	17 to 22,	between wing and leg.
26 to 32,	Leg somites.	23 to 29,	leg.
33 to 35,	Region of cloaca,	30 to 32,	region of cloaca.
36 to 42,	Caudal somites.	33 to 38,	caudal.

The count in the eight-day chick is really an enumeration of nerves. For the four-day chick the exact number of the leg somites was determined by comparison with the enumeration of nerves of the eight-day chick. The wing somites may readily be distinguished at four days, in entire mounts, by their size and by the relatively large amount of mesenchyme formed opposite them. In embryos of more than four days of age the enumeration by nerves is much the easier; and, as the brachial nerves (14, 15, 16) are very much larger than their neighbors (Figs. 3 and 5, *B.P.*), it is simplest in determining the place of a postbrachial somite or nerve to count only the somites back of the last brachial nerve. Thus the postbrachial somites 1–6 are between arm and leg; postbrachial 7–13 are leg-somites, etc.

Experiment 125.

In this experiment a very considerable part of the hind-end was destroyed at the time of appearance of the tail-bud (see operation-diagram), and the embryo was allowed to develop for about four days more (91 hours). The egg was then reopened. The vascular area covered at least three fourths of the yolk. There was no allantois. The embryo lay in large part beneath the blastoderm, but a large aperture in the latter towards the hind-end of the embryo was filled by the amnion through the transparent walls of which the embryo could be distinctly seen. A large part of the blastoderm was removed with the embryo and examination of the under surface was then made in salt solu-

[1] In this enumeration I have omitted again the incomplete anterior cephalic somite.

tion. The amnion was very well distended, and attached round the margins of the somatopleure at the hind-end. Behind this

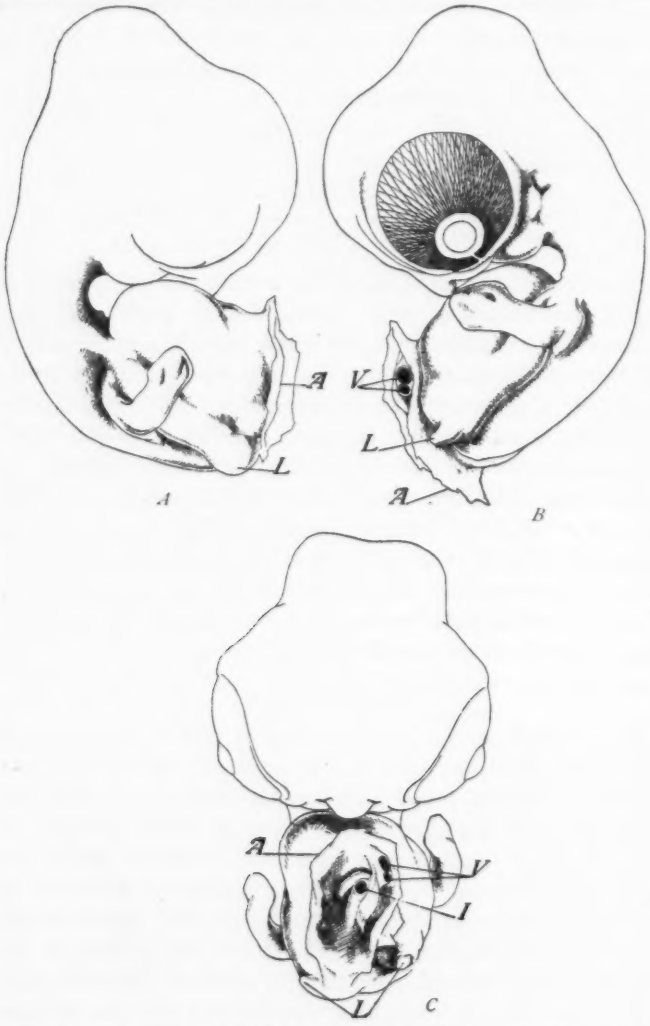


FIG. 2. Three views of a defective embryo (experiment 125) with membranes removed; total age about six days. *A*, amnion; *I*, ectopic intestine; *V*, vitelline artery and vein; *L*, rudiment of hind limbs.

the embryo was naked and there was slight ectopia of the intestine (Fig. 2, *C*).

Figures 2, *A*, *B* and *C* show this embryo from the two sides and from behind. The last figure shows especially well the attachment of the amnion and the ectopia of the intestine external to this. The hind-end of the embryo back of the vitelline vessels is wanting. On the other hand, all parts in front of this are normally developed.

On each side, at the hind-end of the Wolffian ridge, rudiments of the hind limbs are present as prominent conical stumps. From this it would appear at first sight that there has been some regeneration. However the question can be decided only by ascertaining the numerical value of the segments concerned in the formation of these stumps. The embryo was cut into sagittal sections for this purpose.

The enumeration of the post-brachial nerves is the same on both sides, seven pairs being present. The stumps are innervated only by the seventh on each side (Fig. 3). In the normal chick, the nerves innervating the leg are the seventh to the thirteenth postbrachial. Thus it would appear that only one leg somite on each side was uninjured by the operation, and this is the only one that has contributed to the formation of the rudimentary leg. None of the six somites between arm and leg has undergone any alteration of its normal numerical value. This result must therefore be interpreted in the sense of normal self-differentiation of the somites concerned.

Structure of the Leg-rudiments. — But, even though the deficiency of leg somites has not stimulated their immediate neighbors in front to any act of supererogation, it might be that the only leg somite remaining has produced more than its wont. In this connection the structure of the stumps is of interest: Owing to their positions, the left one is cut longitudinally and the right one transversely in the sagittal series. The structure is the same on the two sides (see Fig. 3); there is a single curved rod of precartilage extending out nearly to the tip of the limb; this is surrounded by a mass of dense mesenchyma, evidently premuscle tissue, and externally are the elements of the skin. Now, in the normal limb of corresponding age, the skeleton of the thigh,

crus and pes are separate chondrifications. The crus and pes are thus certainly not represented in the rudimentary hind-limb of this embryo.

In the normal chick the seventh postbrachial nerve innervates only some of the proximal muscles of the leg, those extending from the pelvis to the femur. On the principle that the muscular distribution of the nerve is confined to the derivatives of the corresponding somite, it follows that the seventh postbrachial somite contributes to the formation of only the upper segment of the leg.

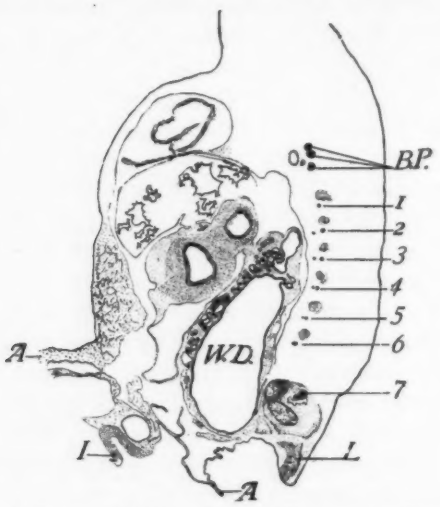


FIG. 3. Sagittal section of embryo shown in Fig. 2, cut considerably to the left of the middle line. *A*, amnion; *B.P.*, brachial plexus (nerves of wing); *I*, posterior end of intestine; *W.D.*, Wolffian duct; 1–7 first to seventh postbrachial nerves.

The absence of the crus and pes is therefore what might be expected if the principle of self-differentiation were rigidly adhered to. We have seen that, as a matter of fact, these are absent in the rudimentary hind limbs of this embryo, so we may conclude, at least, that there is no evidence that the seventh postbrachial somite has produced anything beyond the normal as a result of the absence of the following somites of the leg.

Alimentary Tract. — Practically all of the splanchnopleure of the embryo posterior to the vitelline arteries was destroyed by

the operation. In the resulting embryo, the trachea is a long tube, the lungs are budding out, the œsophagus is well formed, and the stomach is in two divisions, in the anterior of which the glands have begun to form, and the intestine is slightly coiled and opens on the hind surface, across which it is continued as a superficial strip ending in a free blind tag with a slight lumen (Figs. 2*C* and 3). The liver and pancreas are normally formed. In fact the parts present are apparently in the same condition that they would have been had the embryo been intact.

The allantois is entirely absent; and there is no evidence of any compensating growth of the intestine.

Nervous System. — The spinal cord ends bluntly, but the neural canal is prolonged backwards at its ventral angle into a short process which tapers into a bundle of neurites that may be followed a short distance, and then terminates abruptly. It would appear that the descending tracts in the cord have caused the prolongation, and have then pushed out into the adjacent tissues. A similar prolongation appears in all embryos defective at the hind-end (Fig. 3, *A*).

Excretory System. — The Wolffian ducts are much dilated, as there is no outlet for the secretion of the mesonephros (Fig. 3). No metanephric outgrowths have arisen from them, although in the normal embyro of this stage these are well developed. The metanephric region of the Wolffian ducts was of course destroyed by the operation; but, as the ducts are continuous structures, one would not anticipate that the capacity for producing metanephric outgrowths would be limited to a short stretch at the posterior end. Of course another explanation of their absence than that of limitation of potency to a short region of the Wolffian duct is possible, *i. e.*, that suitable predisposing external conditions for their formation are strictly limited (*e. g.*, that their development depends on a certain amount or kind of development of the allantois or cloaca).

Experiment 93.

In this experiment a very considerable portion of the hind-end was cauterized and removed (see operation diagram). Seventy-two hours later the egg was reopened and found living. The vascular area covered about three fifths of the yolk; the circulation was very active, and there was no allantois.

Fig. 4 is a view of the embryo in the amnion, drawn from the under side of the blastoderm. The vitelline blood vessels are attached to the center of the defective hind-end, the entire trunk back of these vessels being absent. The amnion is well distended; its line of attachment is indicated by the dotted line. Just behind this line of attachment is seen a little tag, the hind-end of the intestine. No trace of the hind-limbs is visible externally.

This embryo was cut into sagittal sections. In these there is no trace of the hind limbs, and no evidence that the posterior myotomes or sclerotomes are modified towards the formation of limbs. The embryo is not quite so far advanced in development

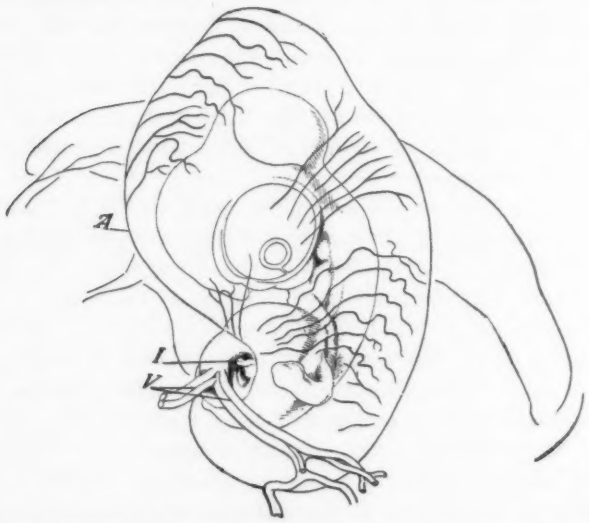


FIG. 4. Defective embryo (experiment 93) in the amnion; part of the folded under surface of the blastoderm is shown. *A*, amnion; *I*, ectopic intestine; *V*, vitelline arteries and veins.

as that of experiment 125, but it is certainly old enough to show the rudiments of the hind limbs or any part of them, were there any tendency towards their formation.

Study of the sections shows that there are only five pairs of post-brachial ganglia and nerves (Fig. 5), the fifth on both sides

being very small, and evidently partially destroyed by the operation. As the first leg-somite is the seventh post-brachial, the absence of rudiments of the hind limbs in this embryo is readily understood.

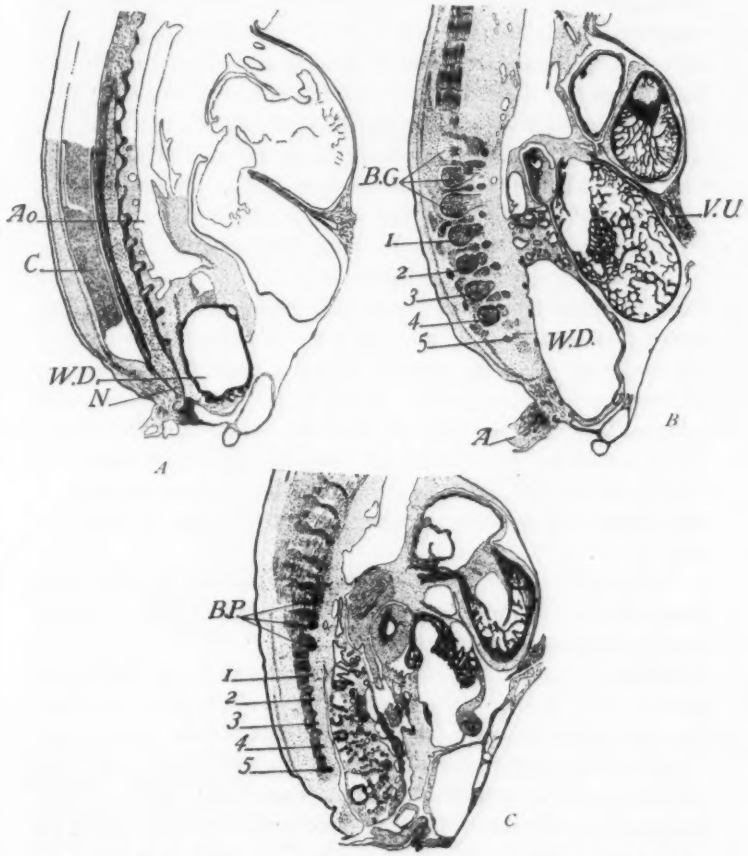


FIG. 5. Three sections from a sagittal series through the embryo shown in Fig. 4. *A*, amnion; *Ao.*, aorta; *B.G.*, ganglia of the nerves of the wing; *B.P.*, nerves of wing; *C*, cord; *N*, notochord; *V.U.*, umbilical vein; *W.D.*, Wolffian duct; 1–5, first to fifth postbrachial ganglia or nerves.

Thus there are two somites less in this embryo than in that of experiment 125. In the latter, which includes one leg somite, rudiments of the hind limbs are formed; in the present case no

such rudiments are formed, although the embryos are very much alike in every other way. It would be interesting to compare an embryo with six post-brachial somites, but I have none yet.

The central nervous system ends bluntly, except for a short prolongation of the ventral angle of the canal (Fig. 5, *A*). This condition, which is characteristic for all embryos defective at the hind-end, appears to be due to growth of fibers in the ventral motor zone, for a bunch of neurites extends back on each side of the ventral middle line nearly to the hind end of the notochord. The notochord extends backwards to the extreme posterior end, thus some distance further than the spinal cord. It thus appears, as in the other embryos of this class, to have undergone some regeneration, or at least growth, at the hind end.

The intestine opens at the posterior end, and is continued as a flat strip along the surface of the splanchnopleure. It is interesting to note that this strip has the same histological structure as the walls of the tube proper, showing that the histogenesis depends upon the character of the cells, and not upon such external factors as the form of the tube. The liver is normal. There is no trace of the allantois; but the stem of the umbilical veins may be seen, in a very rudimentary condition, running in the *septum transversum* to enter the anterior face of the liver (Fig. 5, *B*).

The *Wolffian bodies* are well developed, and the Wolffian duct much enlarged as in other anallantoic embryos (Fig. 5). Metanephric outgrowths are absent.

Summing up, we may say, that all the embryonic rudiments present have differentiated in the normal fashion. There has been no modification of the numerical value of the somites, no compensating growth, and no regeneration, unless we except the elongation of the notochord. Those embryonic organs, whose rudiment or ordinary locality was destroyed, are simply missing.

Experiment 74.

A large part of the hind end was destroyed at the stage of about 52 hours (see operation diagram). The egg was reopened about 68 hours after the operation. The vascular area covered fully half of the yolk; the heart-beat was vigorous.

Fig. 6 gives two views of the embryo. The hind end is entirely absent, and the opening is filled by the enlarged Wolffian ducts. The band of tissue between the two ducts is the hind end of the alimentary tract. The right side is seen to be less developed than the left. In the view from the opposite side, it can be seen that there is a rudiment of the hind-limb on the left side; but apparently none on the right.

This embryo was cut into transverse sections. Study of these shows that there are eight postbrachial ganglia on each side, though the eighth on the right side is smaller than on the left.

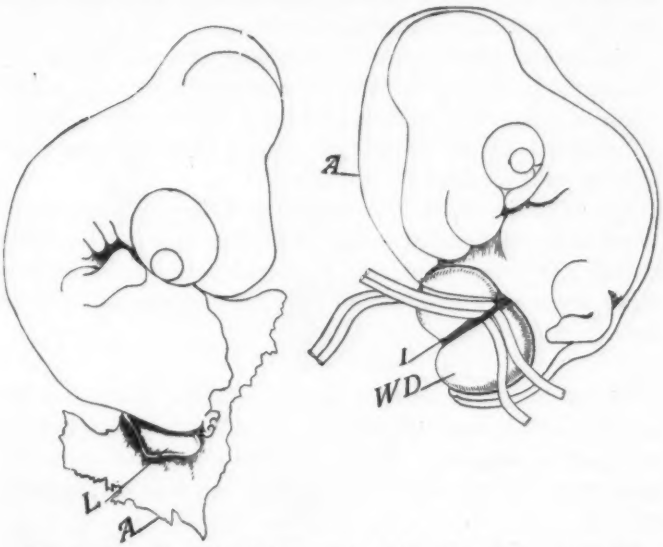


FIG. 6. Two views of a defective embryo (experiment 74). *A*, amnion; *I*, strip of intestinal epithelium; *L*, leg; *W.D.*, Wolffian duct. The vitelline blood vessels are somewhat abnormal.

On the left side the seventh and eighth postbrachial nerves enter the rudiment of the hind limb. On the right side there is no such rudiment, and the seventh and eighth nerves, which are quite large, end bluntly in the mesenchyme, almost as though cut off. The question arises why there is no limb rudiment on the right side? One can explain this by assuming that the operation destroyed the seventh and eighth postbrachial meso-

blastic somites on this side, but not the ganglia and cord of these somites (see Fig. 1). The form of the embryo indicates that this is true in part; but there is a small myotome in the eighth postbrachial somite of this side, and in the seventh the myotome is quite as well developed as on the other side. It would appear probable, then, that the most lateral portions of the seventh and eighth postbrachial mesoblastic somites were destroyed, together with the somatopleure lateral to this; either the rudiments of the hind limb were included in the destroyed parts, or their development was prevented by the scar-tissue. It is impossible to say whether or not the two somites concerned in the formation of the left leg have formed more than the normal.

The central nervous system terminates abruptly, except that the ventral portion of the canal is continued back as a narrow epithelial tube. The notochord extends some sections back of the termination of the neural tube, showing that it has been added to at its hind end since the operation.

The Wolffian ducts are immensely enlarged (see figures). There is no metanephric outgrowth. The allantois is entirely absent. The alimentary canal is normal back to the defective region and terminates in a band lying between the two Wolffian ducts (Fig. 6).

Experiment 18.

The tail-bud of the embryo was just formed at the time of the operation, and it was completely destroyed with a heated needle (see operation diagram). The injury did not extend so far forward as in the other cases mentioned and was asymmetrical (Fig. 1).

Fifty hours later the egg was reopened, and the embryo was found living, the heart beating actively; the vascular area covered about one third of the yolk.

Fig. 7 shows the embryo as it appeared from the under surface of the blastoderm. It will be observed that the amnion ceases with a free edge immediately in front of the hind-limbs; thus the tail-fold of the amnion destroyed by the operation has not regenerated, nor is the allantois visible externally, as it always is in normal embryos of this age. The hind-limbs appear normal; part of the tail at least is present.

The enumeration of the postbrachial nerves in the sections gives the following results: 1 to 6 on each side are in front of the leg-rudiments; the following seven nerves on each side enter the leg-rudiments. On the right side there is only one incomplete ganglion back of the last leg-nerve, *i. e.*, the fourteenth postbrachial; the nerve from this cannot be traced. On the left side there are four complete ganglia and myotomes back of the last leg nerve (14 to 17 postbrachial). Thus, as might be expected, the hind limbs are normal, and the cloacal region is strongly affected.

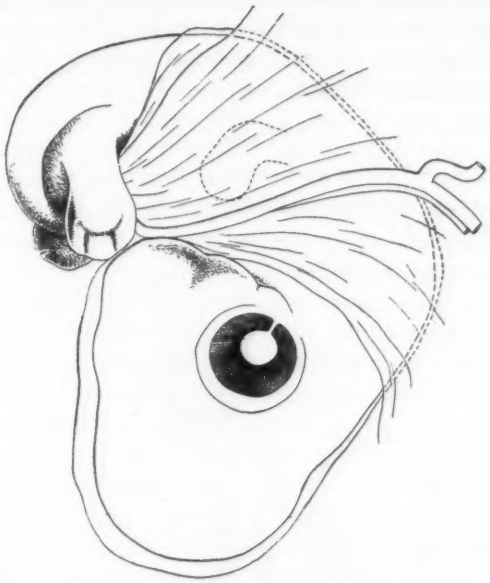


FIG. 7. Defective embryo (Experiment 18).

In the embryo of this experiment the Wolffian ducts have normal relations, but the allantois is thick-walled and undilated; the entodermal cavity of the latter opens out into a groove in the splanchnopleure, leading back to the hind-gut, which is not closed as it normally is; in fact in this embryo the cloaca is the only closed region of the hind-gut; immediately in front of the cloaca it is open ventrally. The stalk of the allantois therefore appears first as a groove on the left side; followed forwards, this

deepens gradually into a canal that penetrates into a mass of mesoblast connected with the somatopleure, and excavates a large irregular cavity in this. The mass extends forwards to the tip of the ventricle and ends in a bifurcated extremity. This represents the allantois, the main mass of which is composed of loose, very vascular mesenchyme forming an appendage to the somatopleure on the left side. On the right side the mesoblast of the somatopleure in the allantoic region is much hypertrophied and forms a large free lobe without any entodermal contents.

It is remarkable that the allantoic rudiments should show such considerable power of growth in the absence of the usual stimulus of internal pressure, which is precluded by the open groove-like connection with the intestine.

Experiment 24.

The hind end of an embryo was cauterized with the aim of destroying the incipient tail-bud and the region of the hind-

FIG. 8. Defective embryo (Experiment 24).

limbs. The egg was reopened 48 hours after the operation. The vascular area covered about two fifths of the yolk, and appeared to be entirely normal.

Fig. 8 represents the resulting embryo from the dorsal surface; the rudiments of the hind-limbs are apparently as well developed as those of the anterior limbs, and the posterior portion of the trunk is separated from the anterior by a sharp depression. The tail turns to the left, and tapers to an end beneath the left hind-limb; part of its course is hidden by a fold of the somatopleure, as shown in the figure. The depression evidently represents the anterior limit of the injury.

This embryo was sectioned. On examination it was found that, although the region behind the depression has the usual structure of the postanal region of the body, its neural tube has no connection with that of the trunk, and the notochord is absent in the anterior third. Mesoblastic somites occur in this region, but the main part of its substance is made up of loose vascular mesenchyme.

It is plain, then, that this part has not regenerated in the sense that it has grown out from the embryo; and there remains only the assumption that its parts have formed from embryonic tissue remaining in this region after the cauterization. The absence of the notochord throughout almost all of the tail shows that the destruction due to cauterization was very extensive; its absence beneath the neural tube for a considerable distance shows, that the neural tube has formed secondarily in this region; because any operation, that would destroy the notochord, would necessarily destroy the neural tube. Farther, the relation of the Wolffian ducts and allantois to the cloaca are abnormal, and the hinder portion of the intestine has no connection with the anterior portion; all of which shows that there was originally complete destruction of tissues through to the entoderm in the anterior part of the cauterized area. There seems to have been in this case a reorganization of the embryonic tissue of the caudal region.

On the left side I find ten postbrachial ganglia back to the region of the defect. Thus at least four segments of the normal hind limb-region are included, viz., 7, 8, 9, 10. The last is small, representing only a fraction of the normal ganglion. On the right side there are eleven full-sized postbrachial ganglia. Thus there are at least one and one half more metameres on the right than on the left side, and this tallies with larger size of the right hind limb and with the operation diagram.

The only interpretation of this result is that the needle destroyed a transverse section of tissue and separated the tail-rudiment from the rest of the embryo, at the same time causing some injury to the tissue of the tail; secondary union then took place. The tissue along the line of junction is peculiar in many respects, including much material, probably remnants of cauterized parts, that takes the plasma stain.

Experiment 187.

In this experiment the operation was made at a stage of about 24 hours; the medullary plate was just formed and the primitive streak was yet long; probably 3 to 5 somites were present (Fig. 9). Three spots were marked with an electrode, one behind the other in the middle line towards the posterior end of the primitive streak. The three spots were at first separate, but later examination showed them running together; so that all of the

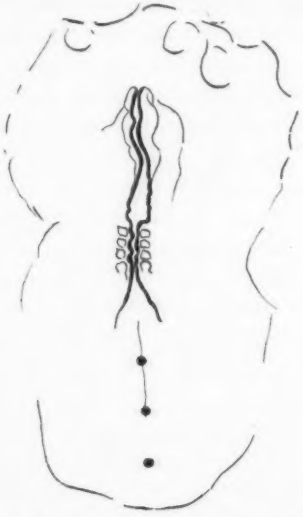


FIG. 9. Operation diagram for experiment 187; drawn from a blastoderm of the same lot of eggs used in this experiment; preserved at the time of the operation.

axial structures were destroyed in this region. The embryo was allowed to develop two days longer and was then preserved.

It will be seen (Fig. 10) that this operation has taken us in front of the vitelline arteries; having been performed before the development of the arteries, it was possible for the rudiment of the vascular system to readjust itself to the new conditions, and establish a vitelline circulation. The vitelline arteries are, however, in about their normal position, *i. e.*, about opposite the 20–22 somites, hence behind the embryo in this case. It is difficult to see why they should have this position and not be shifted farther forward, if the view of His, that the main blood vessels develop from the paths of least resistance in the primitive vascular network, be true.

On the right side of this embryo there are 14 spinal ganglia, and on the left 14 complete and part of the fifteenth; but as, in the fowl, the first two cervical nerves lack ganglia, there are present 16 spinal nerves on the right side, and 17 on the left.

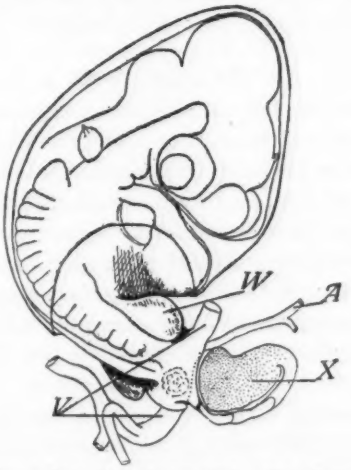


FIG. 10. Defective embryo (experiment 187). The drawing was made from the stained and cleared specimen. *A*, vitelline artery; *V*, vitelline veins; *W*, wing bud; *X*, sac filled with blood, behind the embryo in the prolongation of its axis; this has no connection with the vessels of the embryo.

The normal innervation of the wing is from the fourteenth to the sixteenth spinal nerves. It would thus appear, on the evidence of the nerves, that the operation has destroyed everything back of the wing somites. The wing-buds have a development that appears perfectly normal for this stage.

On the right side there are nineteen myotomes, and on the left twenty and a half. Fig. 10 shows the myotomes of the right side as they appeared in an entire mount of the stained embryo in oil; only 16 can be counted. But sections show, that the large anterior one is really three, and the nineteenth is represented by the mesoblast behind the last complete somite. If we reckon the three anterior somites as cephalic, this leaves sixteen trunk myotomes, and the enumeration agrees with that of the nerves. On the other side of the embryo there is distinctly one more cephalic myotome than on the right side; if, then, we reckon four as the number of cephalic myotomes on this side,[1] there are sixteen and a half trunk myotomes, a result that agrees with the enumeration of the nerves.

The organs in this embryo, as in the others described, have developed normally as far as the cut surface. The Wolffian ducts are enlarged by internal pressure. There has been no compensatory growth and no regeneration, if we except the notochord.

General Discussion.

The chief value of these experiments consists in the fact that they clear the ground for farther researches of a more definite character. It is probable that the results obtained will hold in general for the Amniota, and they may, therefore, be of value in the interpretation of teratological phenomena. Results that I have obtained from destruction of large portions of the brain in young embryos have convinced me that great caution must be exercised in interpreting conditions associated with anencephaly. It should be possible, and I am convinced that it is possible, to produce these various conditions experimentally. Until this is done it seems to me that conclusions, based on teratological material, as to the trophic value of the embryonic nervous system are debatable.

The following questions definitely raised by the experiments just described may be answered here in part:

1. *As to Regeneration.* — The only organ showing evidence of regeneration is the notochord. In all of the embryos described,

[1] Froriep recognizes four cephalic myotomes in the chick; the most anterior, however, becomes rudimentary at a very early stage, and soon disappears; this condition seems to have been attained on one side in this embryo, but not on the other.

the notochord extends a short distance back of the neural tube and its end is very protoplasmic; its further extension in each case is definitely limited by the surface of operation, against which it is pressed (Fig. 5, *A*). One receives the impression that its growth has been stopped by the mechanical hindrance.

One does not expect a vertebrate to show axial regeneration of the trunk, but in certain vertebrates the tail may regenerate. What is the condition in the chick embryo? No. 18 is the only case cited in which any of the caudal somites were left uninjured. In this case there was no regeneration, so far as could be judged.

The very first experiments that I performed on chick embryos were made to determine whether or not the limb-buds might regenerate. The operations were limited to the wing-buds. I found that it was possible to amputate the right one close to the body at a time when its width was equal to or greater than its length, and even later (four or five days); the wound in the amnion might close, and the amputated part remained in the amniotic cavity as evidence of the operation. In the only cases in which the embryo lived for any considerable period after the operation, there was absolutely no sign of regeneration, though the wing-bud of the opposite side increased in bulk several times.

So far as I know, the only evidence, that the organs of the chick embryo possess any different power of regeneration from those of the adult, consists of the above observations on the notochord (tissue regeneration) and of Barfurth and Dragendorff's[1] observation on the regeneration of the lens of the eye from the edge of the optic cup. The last depends on observations on a single case, and in this case the extent of injury to the eye was doubtful. Until the result is confirmed, I believe we are justified in passing it over.

There would remain, then, the general conclusion, subject only to the qualifications already noted, that the embryo of the chick possesses no greater power of regeneration than the adult.

2. *As to the Somites.* — Somites always retain their normal numerical value. But the experiments were not adapted to analyze very accurately the numerous problems presented.

[1] Dietrich Barfurth and O. Dragendorff, "Versuche über Regeneration des Auges und der Linse beim Hühnerembryo," Anat. Anz., Ergänzungsheft zum XXI. Bd., 1902.

Thus the operation destroyed in each case not only definite somites, but also all posterior to the first one injured, and the body wall lateral to the somites. With more refined methods, it may be possible to eliminate single somites from within the series, and to avoid injury to adjacent tissues. Such a technique would enable one to analyze many of the problems offered by the somites: to determine, for instance, the exact part played by them in development of the limbs, the order of origin of the most anterior somites, etc. Such problems are now being studied with partial success, and the results will be published later.

UNIVERSITY OF CHICAGO,
March, 1904.

THE RELATIONS OF THE ANTERIOR VISCERAL ARCHES TO THE CHONDROCRANIUM.

W. K. GREGORY.

The articular relations with the chondrocranium of the upper and lower jaw-cartilages and the hyomandibular, as typified in *Ceratodus*, in *Squalus*, and in *Notidanus*, are in themselves of course generally understood, but comparison of the current definitions and usages of the corresponding terms "autostylic," "hyostylic," "amphistylic" reveals considerable discrepancy, which is highly confusing to the general student; hence the present endeavor to standardize these terms and to give, as far as needed, their synonymy.

From the analysis necessary for the accomplishment of this purpose it has become evident that all the current definitions of "hyostylic," "autostylic" and "amphistylic" are in one way or other unsatisfactory, and that if these conceptions are to retain anything more than historic interest they will have to be extended to include the relations to the chrondrocranium not only of the hyomandibular but also of the hyoidean arch as a whole and of its distal or ventral half, the "hyoid" or ceratohyal. By this extension we are enabled: first, to apply separate and clearly diagnostic terms to the suspensorial conditions of the very phylogenetically separated groups Dipnoi (autostylic) and Holocephali ("holostylic"), hitherto lumped together under the single term "autostylic"; second, to differentiate under the generic concept "hyostylic" four specifically well-marked modes, (*a*) the "hyostylic proper" of typical sharks, (*b*) the "amphyostylic" of *Notidanus*, (*c*) the "euhyostylic" of most rays, (*d*) the "methyostylic" of the teleostomes; third to group all the modern types of suspensorial conditions under the term "cænostylic" in contrast with the ancestral type here called "palæostylic," which is only a few steps below Gadow's[1] suggested form, the "simple autostylic."

[1] "On the Modifications of the First and Second Visceral Arches, etc.," *Philos. Trans.*, Vol. 179 (1888) B, p. 459.

TABLE A.

	PALATOQUADRATE. Proximal or dorsal moiety of "first" visceral or oral arch.	SECOND VISCERAL ARCH.	HYOMANDIBULAR. Proximal or dorsal moiety of "second" visceral or hyoidean arch	HYOID OR CERATOHYAL. Distal or ventral moiety of "second" visceral arch.	DESCRIPTIVE TERMS. Huxley, '76	Weidersheim, '88. (Parker's.)	Zittel, 1902. (Eastman's.)	Gadow, '88	A. S. Woodward, '98.	Preferable Term.
Hypothetical Ancestor.	Preceded by "preoral" arches, gill bearing, not separated by distinct joint from distal (ventral) or mandibular portion. Slight or no cranial attachments.	Intact, and without mid-joint.	Not suspensorial	Not suspensorial.						**Palæostylic.**
Chlamydoselachus. (*Fide* Garman '85.) (Fig. 2.)	Loosely articulating with cranium. With postorbital process.	Intact.	Very large; suspensorial; attached distally to jaws, pointing backward, movably articulating with cranium.	Retaining primitive connection with distal end of hyomandibular, closely applied to and supporting mandible.		Hyostylic.	Hyostylic	Amphistylic.	Hyostylic	**Hyostylic.**
Cestracion.	Closely articulating far forwards with cranium. Deep posteriorly.	Intact.	Of medium size; attached to cranium proximally by ligaments; of considerable suspensorial value; attached to junction of quadrate and mandible.	Articulating with distal end of hyomandibular, attached anteriorly to and supporting mandible.	Amphistylic. Low form of hyostylic.			Autostylic Amphistylic.	Hyostylic.	**Hyostylic.**
Notidanus. (*Fide* Gadow '88.) (Fig. 4.)	Closely articulating with cranium, especially by "otic" process, much deepened posteriorly; inner joint with mandible incomplete. (Gadow.)	Intact.	Slight; proximally attached to cranium by ligaments, of indifferent suspensorial value.	Retaining primitive connection with the distal end of the hyomandibular, attached by ligament to inner superior border of mandible; of slight or no suspensorial value.	Amphistylic.			Autostylic.	Hyostylic.	**Amphyostylic.**
Typica Sharks and *Squatina.* (Fig. 1.)	Loosely attached to cranium.	Intact.	Movably attached to cranium; distally supporting quadrate region, mandible and hyoid.	Retaining primitive connection with distal end of hyomandibular, attached anteriorly to and supporting mandible.	Hyostylic	Hyostylic.	Hyostylic.	Amphistylic. Hyostylic	Hyostylic.	**Hyostylic.** *Sensu strictu.*

Typical Rays. (Fig. 3.)	Free from cranium.	Broken up.	Enlarged, the sole suspensorium	Proximal end, partly by metatrophy of hyomandibular, articulating in different forms at different points along the hyomandibular, finally reaching the cranium, even gaining a distinct articulation with it; widely separated from the jaws; gill bearing. (Wiedersheim, Gadow.)	Hyostylic. Extreme form of hyostylic.	Hyostylic	Hyostylic.	Hyostylic.	Hyostylic.	**Euhyostylic.**
Polypterus. (Fig 6.)	Forming cartilaginous basis of palato-pterygo-quadrate series as in teleosts.	Broken up.	Large, with broad articulation with cranium in sphenotic region, supporting operculum; buttressing metapterygoid and thus, indirectly, the quadrate. Capped by a pharyngohyal (?).	Modifying primitive connection with hyomandibular through intervention of the inter- or stylo-hyal; aiding in support of mandible.	Hyostylic.				Hyostylic	**Methyostylic.**
Acipenser.	Free from cranium.	Broken up.	Enlarged, the sole suspensorium through its separate symplectic segment.	Reduced, beginning the dorsad migration along the hyomandibular towards the cranium.	Hyostylic.	Hyostylic.	Hyostylic		Hyostylic.	**Methyostylic.**
Typical teleosts. (Figs. 5, 7, 8.)	Forming cartilaginous basis of palato-pte ygo-quadrate series.	Broken up.	Articulating with cranium in sphenotic region, distally supporting quadrate through the metapterygoid, symplectic and preoperculum; carrying the whole opercular apparatus, which in turn braces the quadrate.	Modifying its hyomandibular connection by intervention of interhyal. Functioning chiefly in support of tongue and branchiostegal bars.	Hyostylic.	Hyostylic.	Hyostylic.		Hyostylic.	**Methyostylic.**
Amphibia. (*Fide* Gadow.) (Fig. 10.)	Partially fused with cranium, forming cartilaginous basis of dermal bones.	Broken up.	Proximal part functioning as stapes (Gadow).	The proximal end having lost its connection with the hyomandibular generally becomes attached to the cranium as the stylohyal (Gadow).	Autostylic. Amphistylic (of larva).			Autostylic.		**Autostylic.**
Ceratodus. (*Fide* Huxley, '76, Gadow, '88.) (Fig. 9.)	Entirely fused with cranium, forming "suspensorial cartilage" posteriorly.	Broken up.	Reduced, united with cranium anteriorly, bearing the operculum, conical distal portion homologous with the symplectic (Huxley).	Connected by ligament with reduced hyomandibular.	Autostylic.	Autostylic.	Autostylic.	Autostylic Holostylic.	Autostylic.	**Autostylic.**
Chimæra. (See p. 61.) (Fig. 11.)	Entirely fused with cranium. Very short antero-posteriorly.	Intact.	Entirely separated from palato-quadrate and mandible, capped by small pharyngohyal.	Retaining primitive connection with hyomandibular.	Autostylic.	Autostylic.	Autostylic	Autostylic Holostylic.	Autostylic.	**Holostylic.**

The foregoing table summarizes the relations in various forms of the first and second visceral arches to the chondrocranium, and gives the descriptive terms used by authors and the terms here adopted.

Upon this analysis the following definitions may be based.

PALÆOSTYLY: "First" visceral or mandibular and "second" visceral or hyoidean arches retaining in large part their primitive function as gill bearers, subequal in size, with slight or no connections with the chondrocranium; preoral gill arches. Hypothetical.

CÆNOSTYLY: "First" and "second" visceral arches modified in correlation with feeding, unequal in size, one or more of the elements attached to the cranium. Preoral arches changed in function (=labial cartilages and trabeculæ cranii.)

A. HOLOSTYLY[1]: Platoquadrate fused with chondrocranium; second visceral or hyoidean arch intact, non-suspensorial and free from the cranium. Holocephali.

B. AUTOSTYLY[2]: Palatoquadrate fused with chondrocranium; second visceral arch broken up and non-suspensorial, the hyomandibular reduced and united with the cranium. Dipnoi, Amphibia.

C. HYOSTYLY: Palatoquadrate articulating with chondrocranium, hyomandibular more or less suspensorial.

a. Hyostyly proper.[3] Second visceral arch intact, the hyomandibular and hyoid segments forming a movable suspensorium for the upper and lower jaws. Most sharks, *Squatina.*

b. Euhyostyly.[4] Second visceral arch broken up, the dorsal segment (hyomandibular) forming the sole suspensorium, the distal regment (ceratohyal) secondarily, free from all connection with the jaws, functioning solely as a gill bearer. Most rays.

[1] "Holo," in allusion, either to "Holocephali," or to the fact that probably since early Palæozoic times the palatoquadrate and the cranium have formed a continuous whole.

[2] The term "autostylic," hitherto applied to both Dipnoi and Holocephali is here restricted to apply only to the former.

[3] "Hyostyly proper" because the ordinary sharks furnish the traditional type of this condition.

[4] "Euhyostyly," as a progression upon or development of the "hyostyly proper."

c. Amphyostyly.[1] Second visceral arch intact, of slight suspensorial value, much smaller than first arch; palato-quadrate deep posteriorly, articulating by its "otic process" with the chondrocranium. *Notidanus*, *Pleuracanthus*.

d. Methyostyly.[2] Second visceral arch broken up (*i. e.*, with the component segments more or less shifted out of their primitive sequence or relations); symplectic, metapterygoid, pre- and interopercular when present assisting the hyomandibular in the support or bracing of the quadrate and mandible.

The value and permanence of this classification will depend on whether the hyomandibular of teleostomes is homologous, as generally supposed, with that of elasmobranchs. This has been called into question by Pollard ('94) but as his conclusions have not been adopted by subsequent authorities it seems best to accept the traditional view that these elements are truly homologous in the two groups.

The application of these terms in classification and phylogeny is illustrated in the diagram on page 60.

Previous Definitions.

Huxley. — The terms "autostylic," "amphistylic," "hyostylic" were first used by Huxley in his paper of 1876 on *Ceratodus*.[3] The passage in which "autostylic" is defined is as follows (*op. cit.*, p. 40):

"The part of the palato-quadrate cartilage [of *Ceratodus*] which is united with the skull, between the exits of the fifth and second nerves, answers to the "pedicle of the suspensorium" of the amphibian, while its backward and upward continuation onto the periotic cartilage corresponds with the otic process. As in the Amphibia and in the higher Vertebrata, the mandibular arch is thus attached directly to the skull by that part of

[1] "Amphyostyly"; this term retains the element "amphi," so long used (in *amphistylic*) of these forms, while clearly indicating its subordination under hyostyly.

[2] "Methyostyly," in allusion either to the prominence of the *meta*pterygoid in the suspensorium, or to the fact that methyostyly represents a morphological advance upon earlier modes.

[3] *Proc. Zool. Soc. Lond.*, 1876, pp. 24–59. "The Scientific Memoirs of Thomas Henry Huxley," Vol. IV., 1902, pp. 84–127.

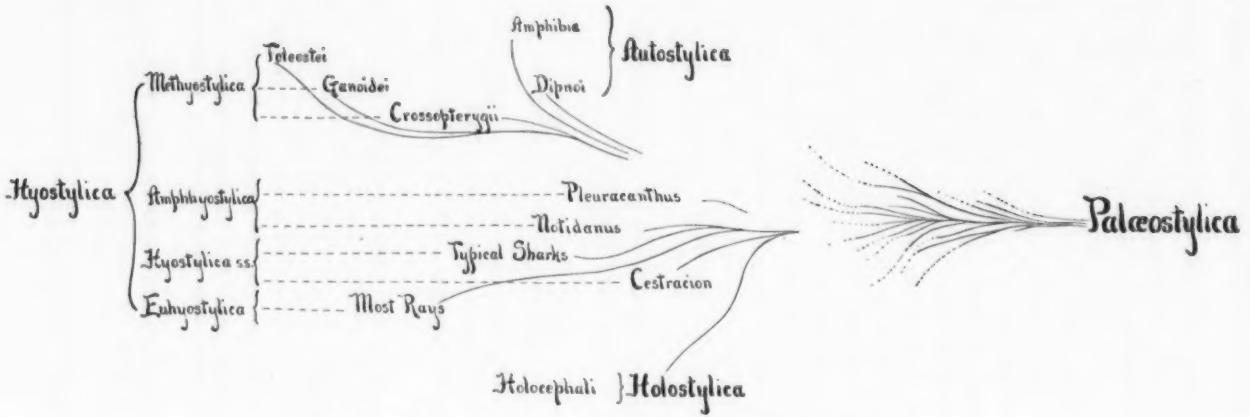


FIG. 1. Application of the newly defined terms to the phylogeny of the fishes.

its own substance which constitutes the suspensorium. It may thus be said to be *autostylic*.

"Among fishes the only [other?] groups which possess an autostylic skull or in which the dorsal end of the mandibular arch is continuous with the cartilage of the brain case are the Chimæroids and the Marsipobranchii."

In this definition of autostyly attention is centered solely upon the relations of the *first* arch with the skull. Huxley notes that in the autostylic *Ceratodus* the hyomandibular is reduced and fused with the skull, but he also uses "autostylic" for *Chimæra* in which the hyomandibular is separate (see above, Table A). For comparison with "hyostylic" and "amphistylic" we may summarize Huxley's description as follows:

AUTOSTYLIC SKULL: *Mandibular or first visceral arch attached to the skull solely by its own dorsal moiety, the palatoquadrate, which is continuous with the skull.*

Huxley describes the HYOSTYLIC and AMPHISTYLIC conditions as follows (p. 41):

"In all other Fishes, except the Marsipobranchii, the mode of connection of the mandibular arch with the skull is different from that which obtains in the Chimæroids and the Dipnoi. The palatoquadrate cartilage is no longer continuous with the chondrocranium . . . but is, at most, united with it by ligament. Moreover the dorsal element of the hyoidean arch, or the hyomandibular, usually attains a large size and becomes the chief apparatus of suspension of the hinder end of the palatoquadrate cartilage with the skull. Skulls formed upon this type, which is exemplified in perfection in Ganoidei, Teleostei, and ordinary Plagiostomes, may therefore be termed *hyostylic*.

"But though the typical forms of autostylic and hyostylic skulls, as exemplified, *e. g.*, by a Sturgeon, a Pike, and a Dogfish or Ray, on the one hand, and *Chimæra*, *Ceratodus*, and *Menobranchus* on the other, are thus widely different, certain Plagiostomes present a condition of the cranium which tends to connect the two by a middle form, which may be termed *amphistylic*."

"In the amphistylic skull the palato-quadrate cartilage is quite distinct from the rest of the skull; but it is wholly, or almost wholly, suspended by its own ligaments, the hyomandibular being small and contributing but little to its support. The embryo amphibian is amphistylic before it becomes autostylic; and, in view of certain palæontological facts, it is very interesting that the link which connects the amphistylic with the ordinary Selachian skull is that of *Cestracion*."

Huxley's conception of hyostyly and amphistyly may be restated as follows:

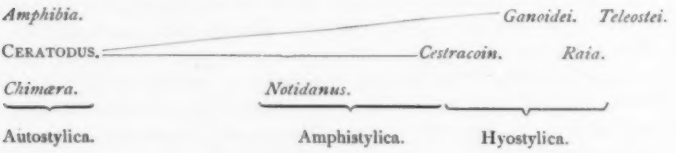
HYOSTYLIC SKULL: *Mandibular or first visceral arch not continuous with the skull in its dorsal portion (the palatoquadrate); the hinder end of the palatoquadrate is chiefly connected with the chondrocranium by means of the hyomandibular or dorsal portion of the hyoidean arch.*

AMPHISTYLIC SKULL: *Mandibular, or first visceral arch attached to the skull wholly or almost wholly by means of its dorsal portion the palatoquadrate, not however by fusion or continuity, but by ligaments; the hyoidean arch contributes but little to its support.*

The Huxleyan conception of amphistyly is that it is in some respects a "middle form," resembling autostyly in the suspensorial self-sufficiency of the first visceral arch, but resembling hyostyly in the lack of confluence of the palatoquadrate with the skull. A similar use of the prefix "amphi" occurs in "*Amphitherium*," which genus was at first supposed to combine mammalian and reptilian characters.

The element "stylic" from *στῦλος*, a pillar, evidently refers to the quadrate region in its architectural relation to the skull. The skull of *Chimæra* seems to have been regarded by Huxley as primitive (so far as I can determine from a careful study of the entire article) and hence in an autostylic skull the palatoquadrate must have been conceived as belonging to the skull: therefore the forces acting upon the jaws in eating would be transmitted to the skull chiefly through the quadrate, *its own pillar* (hence auto, stylic), whereas in the typical hyostylic skull these forces would be transmitted chiefly through the pillar of the hyoidean arch (the hyomandibular) — hence "hyostylic."

Huxley's conception of the phylogenetic relations of the three types of cranial structure are expressed in the following diagram (*op. cit.*, p. 45):



The left-hand column (autostylica) includes the (to Huxley) more generalized types, the most primitive, *Chimæra*, standing at

the bottom of the column; progressive stages of skull structure are represented in the middle and right-hand columns. The cranial plan of *Ceratodus* is regarded as representing the ancestral condition of both *Cestracion* and the Ganoids. *Cestracion*, classed with *Notidanus* as an amphistylic type, is regarded also as transitional, a low form of the hyostylic type.[1] The diagram confirms the inference that Huxley conceived the palatoquadrate as originally a part of the skull, which gradually became constricted off as in *Ceratodus* and finally freed from it entirely as in *Raia* and the Teleosts.

Wiedersheim (*Parker's translation*, '97). —

"The palatoquadrate is usually only united to the basis cranii by ligaments, but in the Chimæroids. . . it becomes immovably fused with it, whence their name of Holocephali. In the Sharks and Rays the palatoquadrate is not directly united to the skull, but is suspended from it by the *hyomandibular*. . . . In this case the skull may be described as *hyostylic*, to distinguish it from *autostylic* skulls, in which the hyoid takes no part in the suspensorium" (*op. cit.*, p. 75).

Comments: (1) "Hyoid" here refers to the hyoidean arch, not to the hyoid or ceratohyal. (2) In general it would be less confusing to say that it is the *mandible* rather than the palatoquadrate which is suspended from the hyomandibular, and that the palatoquadrate rests chiefly upon and is fastened to the mandible.

v. Zittel, 1903 (*Eastman's edition*). —

"In the Holocephali the palatoquadrate and hyomandibular fuse together and with the cranial capsule. The mandible thus becomes autostylic, *i. e.*, articulates directly with the cranium" (*op. cit.*, p. 11).

Comments: (1) The statement in regard to the hyomandibular seems incorrect. In a specimen of *Chimæra collei* kindly loaned to me by Professor Bashford Dean the hyomandibular is seen to be a large independent element (see Fig. 11) serially homologous with the epibranchials as in Selachii, and tipped with a reduced pharyngohyal, both being free from the chondrocranium. (2) To apply the term autostylic to the *mandible* is to introduce a new element of confusion in a matter already sufficiently complicated.

[1] On page 43 of Huxley's memoir the *Cestracion* skull is referred to as a low form of "autostylic type," but examination of the context and of the diagram cited show that this is probably a misprint for "hyostylic."

Parker and Haswell (1897). —

"In some fishes the hyomandibular articulates above with the auditory region of the cranium while the jaws are connected with its ventral end. We may thus distinguish two kinds of *suspensorium* or jaw-suspending apparatus, a *mandibular suspensorium*, furnished by the quadrate, and a *hyoidean suspensorium* by the hyomandibular; in the former case the skull is said to be *autostylic*, *i. e.*, having the jaw connected by means of its own arch, in the latter it is called *hyostylic*; in a few cases an *amphistylic* arrangement is produced by the articulation of both mandibular and hyoid arches with the skull" (*op. cit.*, p. 71).

On page 161 we read that in *Hexanchus* and *Heptanchus*: ". . . there is a prominent post-orbital process of the palatoquadrate for articulation with the post-orbital region of the skull (amphistylic arrangement)."

The definitions cited may be criticised on several grounds:

1. There are several ambiguities latent in these sentences which experience proves to be exceedingly puzzling to the student: (*a*) "jaw" and "jaws" thus used require the most careful analysis to determine which jaws, upper or lower, are intended; (*b*) a *mandibular suspensorium* is no doubt equivalent to "a suspensorium furnished by the mandibular or first visceral arch"[1] but if "mandibular" be mistakenly interpreted as referring to the mandible the very pith of the definition is lost; (*c*) *suspensorium* or jaw-suspending apparatus is understood first with reference to the lower jaw, then to the upper; (*d*) "hyoid" arch is used on this same page (71) as referring to the whole second visceral arch, but on page 161 "hyoid arch" is used of the ceratohyal or hyoid only.

2. The definition of autostylic does not exclude *Notidanus* (which Parker and Haswell themselves call "amphistylic") because in it the hyomandibular takes so small a share in suspension that the suspensorium may be said to be "furnished by the quadrate."

3. The definition of amphistylic as implying the articulation of "both mandibular and hyoid arches with the skull" is exceedingly imperfect. As shown above (Table A, pp. 54, 55) the articulation of both mandibular and hyoidean arches with the skull is not diagnostic of amphistyly since on the one hand both arches

[1] Especially since "mandibular" is used in that sense at the top of the same page.

articulate with the skull in many hyostylic types, and on the other hand in the amphistylic *Notidanus* both arches do not articulate with the skull since the hyomandibular is connected with it only through ligaments (Gadow, '88, Pl. 71, Fig. 1, A).

Gadow (1888). —

Gadow apparently uses the terms in their etymological significance, rather than with the limitations imposed by established usage. Thus he speaks (p. 455) of the "autostylic condition of the *Notidanidæ*" (the "amphistylic" of authors) and applies the same term to the Amphibia (p. 481) and the Dipnoi (p. 459), evidently having in mind the self-sufficiency in all these cases of the first arch as its own suspensorium; so too, "simple autostylic" (p. 459) apparently refers to the ancient and generalized condition from which the modern modes have been derived (cf. "palæostylic," proposed above) and in which the oral arch had not yet begun to borrow support from the hyoidean arch. Again Gadow applies "holostylic" (p. 458) to *Chimæra*, *Ceratodus* and *Cestracion* evidently with reference to the functional ligamentous union of the upper jaw with the cranium in *Cestracion* and to its fusion therewith in *Chimæra* and *Ceratodus*. "Amphistylic" in its etymological sense serves to describe the conditions in "most selachians" (p. 481), in which *both* oral and hyoidean arches are suspensorial, while etymologically the rays are preeminently "hyostylic" (p. 481) since the dorsal element of the *hyoidean* arch, the hyomandibular, is the sole suspensorium (cf. our "euhyostylic," p. 56, *supra*).

But while this bold and highly suggestive use of terms has finally bred in me several clarifying ideas I cannot deny that formerly, wishing to refer to isolated passages of Gadow's work, I experienced a most baffling uncertainty as to his meaning.

Smith Woodward (1897). —

"Among the fishes existing at the present day there may be observed two distinct plans of cranial structure, between which no definitely intermediate conditions can be recognized. In *Chimæra*, *Protopterus*, *Ceratodus* and their allies, the upper segment of the mandibular arch is directly fused with the chondrocranium, while the corresponding segment of the hyoid arch is atrophied or absent; in the Elasmobranchs and the so-called "Ganoidei" and "Teleostei" the same elements are loosely articulated with the chondrocranium, the upper segment of the hyoid arch forming a

movable suspensorium. The first condition is now commonly known as the *autostylic*, and the second as the *hyostylic*."

Comments. — This passage is perfectly clear and illuminating. It adheres closely to the essential features of Huxley's conceptions, and it makes a decided advance beyond them not only in stating clearly that between modern autostyly and hyostyly "no definitely intermediate conditions can be recognized," but also in implicitly reducing the amphistylic mode to its true place as a special phase of the hyostylic — a suggestion which has been followed in the present paper (see above, pp. 57, 59).

But not even these definitions, I think, will survive. They fail to emphasize the differences between the autostyly of Dipnoi and the autostyly (here termed "holostyly") of Chimæroids; nor do they indicate that by taking also the distal segment of the hyoidean arch into account we may place the whole subject upon a new, and apparently phylogenetic basis.

In conclusion I desire to thank Professor Henry Fairfield Osborn and especially Professor Bashford Dean for various courtesies and suggestions.

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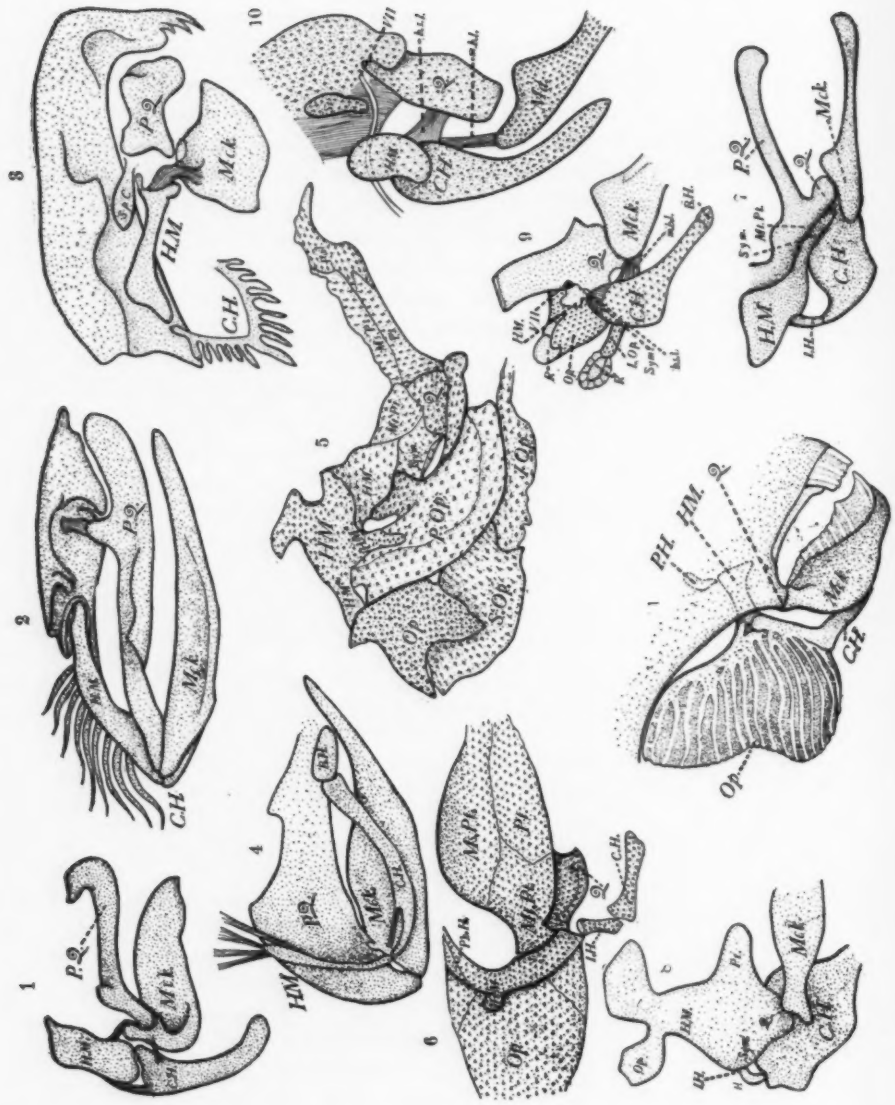
1
P.Q.
Mck.
H.M.
2
P.Q.
Mck.
C.H.
3
H.M.
P.Q.
Mck.
C.H.
4
H.M.
P.Q.
Mck.
C.H.
5
H.M.
P.Op.
Op.
Q.Op.
Q.R.
6
Mck.
M.Pt.
Pt.
C.H.
Op.
7
Pt.
H.M.
Mck.
C.H.
Op.
8
P.Q.
Mck.
H.M.
C.H.
9
Mck.
C.H.
H.M.
Op.
10
C.H.
Mck.
1
P.H.
H.M.
Mck.
C.H.
Op.
2
P.Q.
Mck.
C.H.
H.M.

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COLUMBIA UNIVERSITY,
DEPARTMENT OF ZOÖLOGY,
February 10, 1904.

PLATE I.

EXPLANATION OF ABBREVIATIONS.

P.Q., Palatoquadrate.
Mck., Mandible (Meckelian cartilage).
H.M., Hyomandibular (epihyal).
B.H., Basihyal.
C.H., Ceratohyal.
P.H., Pharyngohyal.
Sp.C., Spiracular cartilage.
I.H., Inter- or stylohyal.
Pal., Palatine.
Ms.Pt., Mesopterygoid (entopterygoid).
Pt., Pterygoid.
Mt.Pt., Metapterygoid.
H.M'., Anterior limb of hyomandibular.
H.M''., Median limb of ditto.
H.M'''., Posterior limb of ditto.
Sym., Symplectic.
Q., Quadrate.
P.Op., Preoperculum.
I.Op., Interoperculum.
Op., Operculum.
S.Op., Suboperculum.
VII., Aperture for seventh nerve.
h.s.l., Hyosuspensorial ligament.
m.h.l., Mandibulohyoid ligament.
St., Stapes.

Cartilaginous elements stippled, osseous elements with small crosses.

FIG. 1. "Hyostyly proper." *Centrophorus granulosus.* Embryo. After Gadow.

FIG. 2. "Hyostyly proper." *Chlamydoselachus anguineus.* After Garman.

FIG. 3. "Euhyostyly." *Trygon* sp. After Gadow.

FIG. 4. "Amphyostyly." *Heptanchus cinereus.* Internal view. After Gadow.

FIG. 5. "Methyostyly." Diagram of the relations of the suspensorial and opercular regions in the cod.

FIG. 6. Methyostyly. *Polypterus.* View from within and below. The metapterygoid is supported by the hyomandibular.

FIG. 7. Cartilaginous basis of methyostylic arrangement in the young salmon. After Parker and Bettany.

FIG. 8. Cartilaginous basis of methyostylic arrangement in a larval siluroid. After Pollard. All the cartilages of the suspensorial region seem to have secondarily coalesced.

FIG. 9. "Autostyly." *Ceratodus.* After Huxley. *R*, *R'*, vestigial hyoid rays. The operculum is borne by the reduced hyomandibular.

FIG. 10. "Autostyly." *Proteus.* After Gadow. The stapes represents the upper portion of the hyomandibular (Gadow).

FIG. 11. "Holostyly" *Chimæra collei.*

VARIATION IN BEES — A REPLY TO MR. LUTZ.

EVERETT F. PHILLIPS.

In the December (1903) number of this journal there appeared an article entitled "Comparative Variability of Drones and Workers of the Honey Bee" by Dr. D. B. Casteel and myself in which we concluded that the drones show the more variability, which result we attributed to the effect of the size of the cells on the developing larvæ and pupæ. Mr. Frank E. Lutz in the April (1904) number of this BULLETIN thinks it "well worth while to consider a few points about the paper" and concludes that we have not proven our point. I desire here to point out the errors in his manner of dealing with our measurements and his methods of drawing conclusions and to defend our position.

The fact that in the consideration of abnormal veins we found that "there are 206 irregular drone wings and 30 irregular worker wings or almost seven times as many for drones as for workers" points rather conclusively to our position in regard to comparative variability. Our statements in regard to coloration are also disregarded, evidently because there were no figures given and therefore they were not considered worthy of mention. In another paper[1] I have dealt with the comparative constancy of the color areas of the two sexes and there offer evidence which confirms the position maintained in our paper, although as there used it was intended to prove an entirely different point. In quoting from a letter from Mr. E. R. Root, a high authority in apiculture, it was there stated: "The drones from these queens (imported Italians of pure stock) varied greatly in their markings. Some of their sons would have a great deal of yellow on them, while others would be quite dark. . . . *Bees* (workers) from these queens were all uniformly marked." In variation work but few investigators take the trouble to study more than one character yet here are two which are rather conclusive and on these we would be willing to rest the case.

[1] "A Review of Parthenogenesis," *Proc. Am. Philos. Soc.*, XLII., No. 174; *vide* pp. 277–8.

I will now take up the more definite criticisms of Mr. Lutz. In our paper we gave reasons which to us seemed good why we did not employ the standard deviation method in our work, stating distinctly that "that would be undesirable except with far more measurements"; this could be interpreted only as a desire on our part to avoid a high probable error and no one need "wonder greatly" why we did not employ this simple test since we distinctly stated that we did not think it desirable, giving full reasons for such a decision. It was not our intention to take exception to the methods of the workers in variation for I believe that the results of variation work should be stated in mathematical formulæ where such a thing is possible without too great a "probable error." If our critic is trying to defend such methods under the impression that we combat them, he is laboring under a misapprehension. However Mr. Lutz has seen fit to figure out the standard deviation and probable error for two of our tables and concludes "that the differences between the two sexes, as shown by these data are of no significance." I am still of the opinion that it is unwise to use these methods for so few individuals on account of the large probable error but since this is the way in which our results are questioned let us examine the figures and see if there is not "great danger that, having collected a set of measurements" our critic makes "a show of accuracy that will lead" him "and others astray by reason of careless and insufficient analysis." For ease of reference I give the results of Mr. Lutz.

The argument of Mr. Lutz is that since there is as much difference in standard deviation between various lots of drones as there is between the averages of the standard deviations of drones and workers, our conclusion that drones vary the more is false. But is it not evident that in every case of the drones (except lot II.) the standard deviation or index of variability is greater than that of the workers? I cannot see what bearing the differences in the standard deviation of the various lots of drones has on the question since in every case except one, the drones do vary more than the workers; and this it was that we attempted to prove. In regard to this lot II. we said: "The drones in lot II. were taken from a hive in which there were no drone cells except

HOOKS ON HIND WING.

Lot.	Number of Specimens.	Standard Deviation.	Probable Error.[1]
Drones.			
I.	50	2.1548	0.1453
II.	100	1.5435	0.0736
III.	100	1.7716	0.0845
IV.	100	1.6486	0.0786
V.	50	2.0988	0.1416
VI.	98	1.9377	0.0934
Workers.			
I.	50	1.5223	0.1027
II.	350	1.5564	0.0397
III.	100	1.5523	0.0740

VEIN *R*.

Lot.	Number of Specimens.	Standard Deviation.	Probable Error.
Drones.			
I.	50	2.4023	0.1620
III.	100	2.9598	0.1412
V.	50	2.2517	0.1519
Workers.			
I.	50	1.5637	0.1055

possibly a very few in the corners of the frame or near the top bar of the frame since all the combs were made on what bee-keepers call foundation and the cells were uniformly of worker size. These drones show the least variation since they were all hatched under the same conditions." Even if we add the probable error to the standard deviation of workers and subtract it from that of the drones this result holds except in lots II. and IV. and it would seem that from these figures (made by Mr. Lutz himself) that our results are most strongly confirmed.

The additional criticism is made that we lumped the different lots together because they seemed to be alike " when really their only claim to homogeneity is that they are of the same sex and all bees — Italians, hybrids, 'peculiar strains,' et al., from central Ohio to eastern Pennsylvania being jumbled together." This

[1] In discussing the average standard deviation of this table Mr. Lutz takes the average of the probable error for the lots of 50 and 100 without taking into consideration that the probable error should be smaller for 500 than for these lots. Surely since all the lots show the same greater variability of the drones (except lot II. as explained later) the probable error is considerably smaller than that given by Mr. Lutz.

was done only in the case of our figures concerning the ratios between the veins M_2 and m where a careful examination showed us that these ratios did not vary according to lots but from the criticism it might be inferred that we had committed this "grave error" throughout the work. Just how Mr. Lutz could know, since the ratios for the individual wings were not published, that we had "jumbled together" some measurements in an unjustifiable manner is still a mystery but I am convinced that his criticism is unjust, having carefully examined the ratios with this very criticism in mind at the time of the preparation of our paper.

At the close of his article our critic says: "It is also probably unnecessary to remark that, even if it turns out that the greater variability of the drones can be established, their proof of their theory to account for this difference seem rather unsatisfactory." I think it is shown, by us and by Mr. Lutz, that the drones do vary the more and our theory of the cause, based as it is on careful investigations of the habits of the bee, must be controverted by more observations of an equally careful nature. Too often students of variation work on forms, concerning the habits of which they know nothing, and conclusions are reached which would be modified if causes were looked for in the habits, but I feel free to state that we are not open to that criticism.

By omitting the parts of our paper in which we explain our stand regarding the variability being "due to chance," Mr. Lutz would make out that we do not know that all chance is in accord with some mathematical formula. On this point we stated: "It may be argued that variation according to chance is but a way of stating our ignorance of the true law, but if there is a law for this variation it is certainly very obscure, and the working out of this law would require an extremely large number of measurements taken from individuals each one with its life history known," and again: "While it is probable that even this chance is according to fixed law, the fact remains that in any event this law is beyond a possibility of formulation from any observations except those extending over far more individuals than those here used." If as we believe the particular size variation of any individual bee depends on the size of the cell in which it grows, then the formulation of this law of variation must be

based on the law which governs the queen in moving over the comb and in choosing where she shall lay her eggs and on the law which governs the bees in cell building. We leave it to our critic to formulate these laws since we confess to a lack of mathematical ability for any such problems.

Finally we acknowledge the mistake in averages mentioned by Mr. Lutz but can only state that we believe this to be the only correct criticism of our paper which he has made.

Zoölogical Laboratory,
University of Pennsylvania.

NOTES ON A PECULIAR ACTINOZOAN LARVA.

L. R. CARY.

For a number of years past a few specimens of a large trochophore-like larva have been taken each summer in the tow near Beaufort, N. C., but they have never been seen to transform.

While at the laboratory of the United States Fish Commission at Beaufort,[1] during the past summer, I had the good fortune to secure a number of these peculiar larvæ.

The larvæ were taken while towing outside the harbor on August 15, after a heavy southeast storm which had continued for two days, and which had driven in shore specimens of several forms not usually found inside the Gulf Stream.

These larvæ were taken to the laboratory and at the suggestion of Dr. Caswell Grave, of the Johns Hopkins University, were put in aquarium jars of sea-water containing sand rich in diatoms. By this method they were kept alive for the remaining seven weeks of my stay at the laboratory.

The larvæ, Figs. 1 and 2, are elongate-oval in shape when in an active state, changing to a very nearly spherical form when they are disturbed. They are from two to four millimeters in length, and of a light brown or cream color.

At a point about one fourth of the distance from its anterior end, the body is encircled by a ridge which lies at the bottom of a shallow groove. This ridge bears on its surface two parallel bands of long stiff setæ, Fig. 4. The setæ-bearing ridge is not continuous around the body, but on one side it is interrupted and the ends overlap for a short distance, Fig. 1.

The whole surface of the body is provided with a covering of short cilia which are the true locomotor organs. The function of the bands of setæ is not apparent. They move only at irregular intervals, and then the force of their movement is in a direction antagonistic to the progress of the larva.

[1] I am indebted to the Hon. G. M. Bowers, U. S. Commissioner of Fisheries, for the privilege of occupying a table at the Beaufort Laboratory, and to Dr. Grave, the director, for many favors received while there.

At both the anterior and posterior ends of the body there is a depression lined with cilia. The depression at the anterior end has at its bottom an opening communicating with the interior.

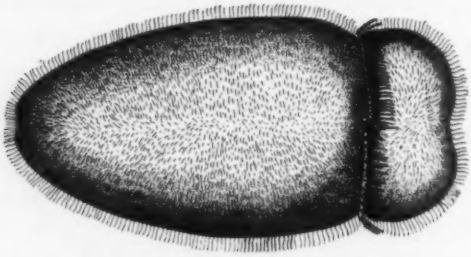

FIG. 1. Larva in the active expanded condition, × 30.

In swimming, the larva rotates rapidly on its long axis, and the anterior end describes a small circle about the axis of progression so that the larva advances by a kind of corkscrew

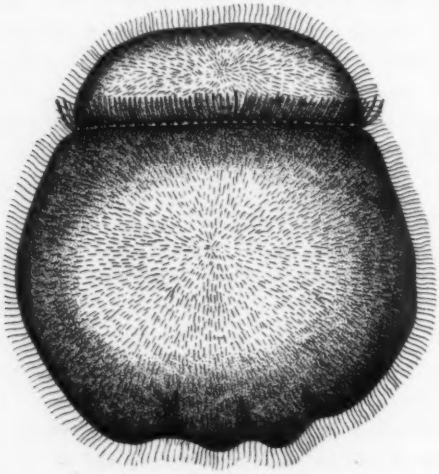

FIG. 2. Same larva as in Fig. 1, when contracted, × 45.

movement, such as has been described for the larva of *Astroides* by Lacaze Duthiers[1] and for *Renilla* by E. B. Wilson.[2]

[1] Lacaze Duthiers, H., "De Devellopment des coralliaires," *Archiv Zool. exper. et gen.*, Tom II., 1873.

[2] Wilson, E. B., "The Development of *Renilla*," *Phil. Trans. Roy. Soc. London*, Vol. CLXXIII., 1883.

The habits of the larva differ from those of the larvæ just mentioned in that it does not come to the surface of the water in the aquarium, nor does it remain motionless for any appreciable time unless it is disturbed, when it contracts and sinks to the bottom of the vessel.

Two of the larvæ went through their transformation two days after they were brought into the laboratory. When transformation is to take place, the larva settles down and becomes attached by the anterior end. In a short time the tentacles are budded out from the upper (posterior) end, and the mouth opening

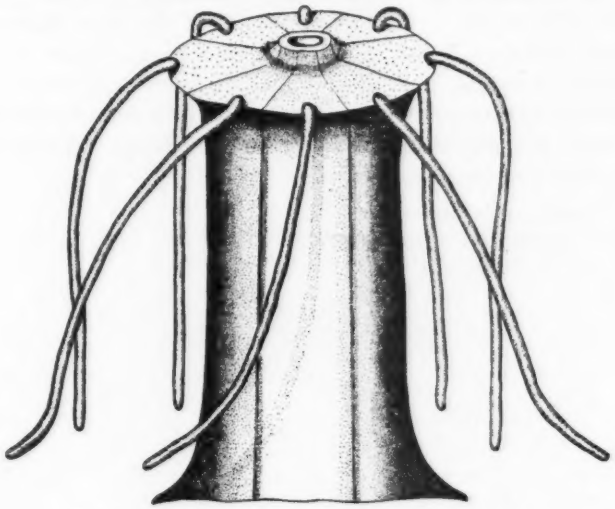

FIG. 3. Young polyp, two days after transformation, × 60.

breaks through the body wall within the circlet of tentacles. Within twenty-four hours from the time of the attachment at the beginning of the transformation, the young polyp had assumed a form such as is shown in Fig. 3.

In the five or six weeks after transformation, during which the young polyps were under observation, there was no apparent external change other than a gradual increase in the size of the animal as a whole without any change in proportions.

All the larvæ, with one exception, had transformed by Sep-

tember 7, three weeks after they were secured. The other specimen had not transformed on October 3, and, as far as could be determined, it had undergone no changes in size or form.

FIG. 4. Diagram to show position of setæ.

The actinian, of which the larva just described is the immature form, is not definitely known to me at present, but a number of mature sea anemonies, probably of the genus *Amophyllactis*, were cast upon the beach near where the larvæ were found during the same and subsequent storms coming from the same direction. Since both these forms appear only after heavy storms from a definite direction, and since there are certain structural resemblances between the young polyps and the mature actinians, it seems not improbable that they may be different stages in the life history of the same species.

ZOOLOGICAL LABORATORY,
JOHNS HOPKINS UNIVERSITY,
March, 1904.